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CONTENTS

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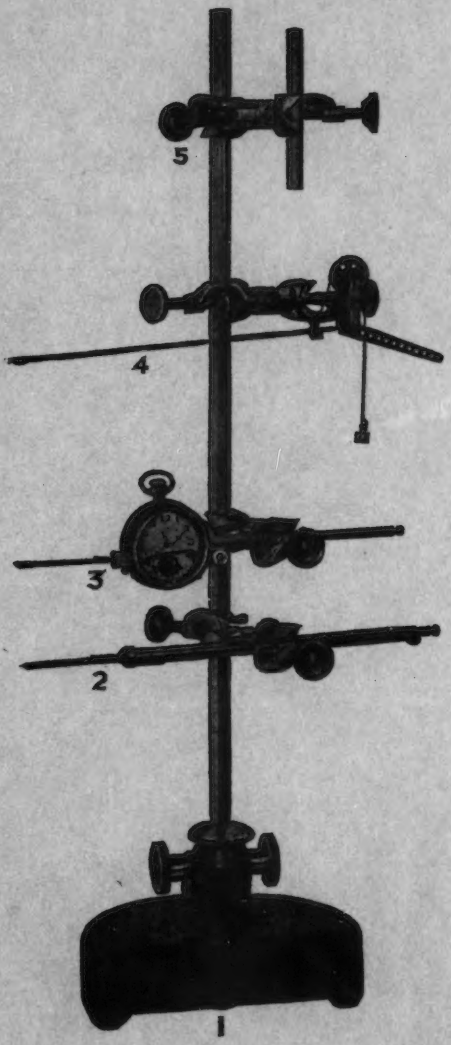

JOSEPH BECKER

437 West 59th Street **New York City**

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STUDIES ON VIGOR

IX. Erographic Studies on Adrenalectomized Animals

HOWARD M. GANS and HUGH H. MILEY

From the Department of Physiology, The Ohio State University

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One of the cardinal symptoms of Addison's disease is marked muscular weakness. The patient suffers from an almost complete indisposition for any exertion and is very easily fatigued. A short series of movements can be made with some energy, but fatigue almost immediately follows (Addison, 1885).

Mosso's ergograph is frequently used as a means of differential diagnosis. An individual with a true Addison's complex always shows a characteristic ergographic record. The contractions are of normal height at first, but very rapidly diminish and within a few minutes complete fatigue develops (Langlois, 1892).

Durrant (1924) studied the effect of adrenal extirpation on the voluntary activity of the albino rat. In a series of 24 experimental and 16 control animals he found a marked diminution in the voluntary activity of the experimentals over a period of 7 weeks. By extending the experimental period to 16 weeks it was found that 7 rats completely recovered within 35 days, 10 partially recovered and the rest maintained a marked adynamia throughout the entire period.

We have attacked the same problem by the use of ergographic tests in normal and adrenalectomized albino rats. The results substantiate the previous work and put on an experimental basis the etiology of Addison's disease. These results show, we believe, beyond any possibility of a doubt, that adrenal hypofunction results in the muscular adynamia characteristic of the syndrome. The total work performed by the adrenalectomized animals before complete fatigue was only $\frac{1}{16}$ of that of their controls.

Methods and observations. Male and female albino rats of approximately the same age (90 to 360 days) and weight were used in these ex-

TABLE 1

Showing results of ergographic tests on adrenalectomized animals and their controls

ANIMAL NUMBER	AGE	INTERVAL AFTER ADRENALECTOMY	WEIGHT OF ANIMAL	WEIGHT OF MUSCLE	ABSOLUTE STRENGTH	ABSOLUTE STRENGTH PER GRAM	WORK	WORK PER GRAM
	days	*days*	*grams*	*grams*	*grams*		*grams cm.*	*kgm. cm.*
1E	105	10	142	1.0	230	230	75,000	75.0
2E	120	11	145	1.0	170L	170	4,500	4.5
3E				1.2	260R	216	6,300	5.2
3E	100	4	140	1.0	350	350	18,000	18.0
4E	120	4	148	1.2	420	350	42,000	35.0
5E	90	5	125	0.9	450	500	10,500	11.6
6E	126	2	164	1.1	260	236	108,000	98.1
7E	120	1	160	1.1	310	281	101,220	92.0
8E	126	7	165	1.3	400	307	7,700	5.9
9E	126	1	165	1.1	360	327	1,350	1.2
10E	114	6	150	1.4	360	257	72,000	51.3
11E	150	3	184	1.3	340	261	52,500	40.3
12E	120	11	175	1.6	230	144	129,500	80.9
13E	131	5	174	1.3	400	307	9,900	7.6
14E	124	4	170	1.4	310	221	288,960	206.4
15E	124	2	172	1.4	470	335	234,000	167.1
16E	175	4	190	1.3	250	192	96,000	73.8
17E	175	1	207	1.2	400	333	5,040	4.2
18E	137	1	214	1.6	720	450	146,520	91.5
19E	360	8	302	1.7	630	370	315,000	185.2
20E	268	3	250	1.7	460	270	156,750	92.2
21E	344	8	275	1.8	500	277	16,200	9.0
22E	177	5	235	1.6	440	275	34,200	21.3
23C	120		145	1.2	290	241	867,796	723.1
24C	126		161	1.3	140	108	568,450	437.2
25C*	138		180	1.0	340	340	614,400	614.4
26C	160		188	1.3	240	184	2,663,900	2,049.1
27C†	131		176	1.3L	420	323		
				1.4R	600	428	840,000	600.0
28C	175		196	1.0	200	200	174,990	174.9
29C	181		193	1.5	340	226	646,340	430.9
30C†	175		200	1.5	540	360	3,279,000	2,186.0
31C	175		200	1.5	330	220	1,355,800	903.8
32C*	137		220	1.4	690	492	819,000	585.0
33C	171		219	1.4	280	200	1,621,418	1,158.1
34C	171		214	1.4	220	157	1,006,369	718.8
35C†	177		226	1.6	510	318	1,980,000	1,237.5
36C	177		256	1.9	520	273	4,024,400	2,233.8
37C	177		226	1.8	350	194	2,205,671	1,225.3
38C	170		252	1.6	380	237	1,843,570	1,152.2
39C	177		243	1.6	280	175	1,398,250	873.9
40C	142		282	1.4	410	292	1,347,053	962.1
Averages, controls:	160		209	1.4	382	272	1,514,197	1,015.3
Experimentals	156	4.8	184	1.3	379	290	83,962	64.2

* Adrenal rests found at autopsy.

† Sham operation performed.

E, adrenalectomized animals; C, control animals; L, left gastrocnemius; R, right gastrocnemius.

periments. Many of these were litter mates. Experimental and control animals of the same sex were used in each case. Whether litter mate controls have any special value in this type of experimentation, however, is doubtful. Many observations in this laboratory indicate that the variation in activity within litters is considerable.

One-half of the animals used were kept in revolving cages and were under daily observation throughout the period of the experiment. After a period of approximately 20 to 30 days the more active animals were adrenalectomized. The rest were used as controls.

The technique used in adrenalectomy was briefly as follows. The animals were anesthetized with ether; an area on the back extending from the lower rib margin downwards for about 6 cm. was clipped and then shaved. This area was then washed with 1 per cent lysol solution and painted with iodine. A mid-dorsal incision of about two centimeters was made through the skin and the fascia was separated from the muscle by blunt dissection. The skin was then reflected to one side and with fine curved scissors a small puncture was made at a point about 5 mm. below the lower rib margin and 15 or 20 mm. laterally from the mid-dorsal line. The blades were then separated thus making a narrow slit. Using fine curved forceps the adrenal body was delivered and removed. A similar procedure was performed on the opposite side. The stab puncture through the muscle was quite small and no stitches were taken. The skin incision was then closed with silk and a small gauze and collodion dressing applied over the wound. The time, from making the incision until the suture was completed, averaged seven minutes.

Lewis (1923) has stated that adrenalectomized animals became sensitive to cold immediately after operation. Experiences in this laboratory have indicated that mortality following adrenalectomy is diminished if the rats are kept at a fairly high temperature. For this reason the animals were kept in a warm room and the mortality apparently was reduced.

Sham operations were performed upon some of the controls. Such an operation consisted of the previously outlined procedure except that the adrenals were merely picked up and returned to their positions.

In determining the absolute strength and fatigability, the technique described by Gans and Hoskins (1926) was used. The animals were anesthetized with amytal. The sciatic nerve was laid bare, sectioned near the hip joint, and adjusted in a Sherrington electrode. The skin was then sutured over the incision. A medial incision was made into the thigh; by blunt dissection the femur was exposed and fixed by means of a modified Harvard femur clamp. The gastrocnemius muscle was isolated from its distal attachment and connected by a strong linen thread to a heavy type Harvard muscle recording lever. The approximate absolute strength of the muscle was first determined, using single break

shocks of a maximal faradic current as stimuli. In order to obviate fatigue this orienting study was confined to four trials of each muscle. An initial weight of 250 grams was attached and the nerve stimulated. Using the first reaction as a guide, lead weights of the estimated required number were either added or removed, and the stimulus repeated. Four trials were adequate to fix the limit rather accurately.

The muscle was then afterloaded with a weight of 100 grams and stimulated automatically at one second intervals by means of a faradic current, using break shocks only. The make stimuli were short circuited by means of a special device which Gans and Hoskins have illustrated and described. The contractions were registered by means of a slow-moving extension kymograph.

After completion of the ergographic tests a post-mortem examination was made of each animal and a careful search was made for adrenal rests. In two rats small portions of one adrenal body were found. This was verified by histological examination. In both cases the animals gave fatigue records similar to those of the controls.

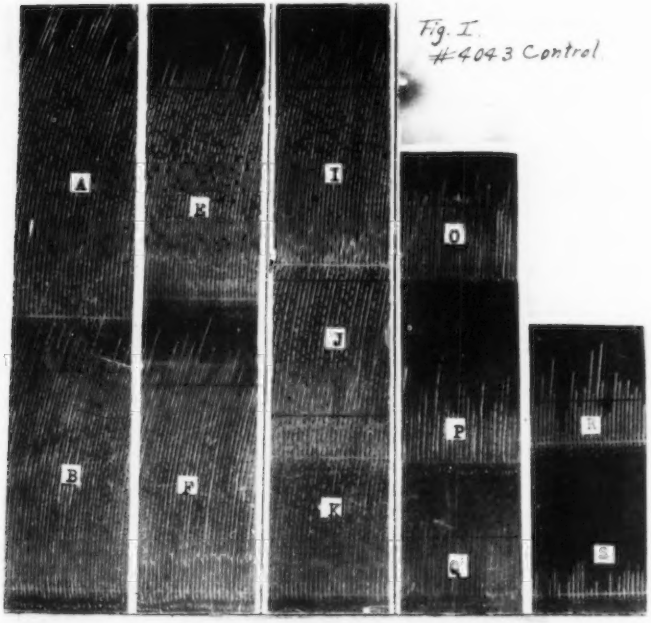
The fatigue records obtained from the experimentals are quite characteristic. The height of the initial contractions compares favorably with that of the controls, being even higher in some cases, but there invariably occurs a progressive rapid diminution in height, and complete fatigue develops in from 10 to 60 minutes, whereas in the controls, the initial height is sustained for several hours and fatigue sets in in a variable period of from 8 to 26 hours. This difference is so great and so striking that if the muscle of an adrenalectomized rat continues to show a good height of contraction after an hour on the ergograph, the prediction may be made that at autopsy adrenal rest will be found.

In all, 22 experimental and 18 controls were used. The average total amount of work recorded by the controls was 1015.3 kgm. cm./gm.

One experimental animal, no. 14, did 206.4 kgm. cm./gm. of work, while no. 28, a control, did 174.9 kgm. cm./gm. No. 19, another experimental, did 185.2 kgm. cm./gm. of work. Of the remaining rats the controls greatly exceeded the experimentals in the amount of work done. No allowance was made for friction of the recording apparatus hence the actual amount of work was greater than that indicated.

The interval between adrenalectomy and ergographic tests varied from one to eleven days. The experimental animals lost weight during the post-operative period as compared with their controls but in the experimentals the muscle weight was apparently not affected.

The average absolute strength of the experimental rats was 379 grams while that of the controls was 382 grams. Computing this per gram of muscle substance the average absolute strength per gram of muscle in the experimental animals was 290 grams while that in the controls was 272



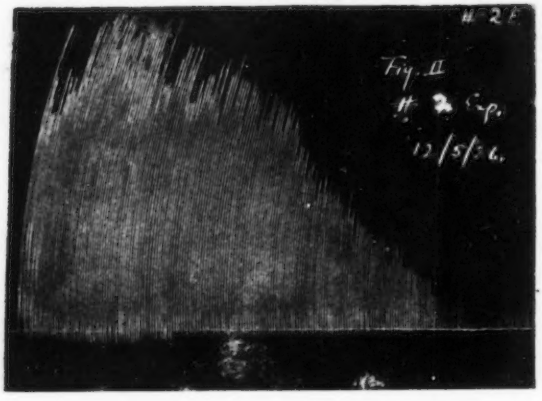


Fig. 1. Ergograph of normal animal. Rat 4043. Total work period, 18 hours; work, 2,205,671 gm.cm. *A*, beginning of test; *B*, after 1 hour; *E*, after 4 hours; *F*, after 5 hours; *I*, after 8 hours; *O*, after 14 hours; *R*, after 17 hours; *S*, after 18 hours.

Fig. 2. Ergograph of adrenalectomized animal. Rat 2 E. Total work period, 4 minutes; work, 4500 gm.cm.

grams. This difference may perhaps be explained by the fact that the more vigorous animals were selected for adrenalectomy.

The marked fatigability observed in the experimental animals following adrenalectomy can be very well correlated with the muscular adynamia observed clinically. The fact that the absolute muscle strength in these animals is apparently not affected by adrenalectomy adds some weight to the conception that certain toxic products of metabolism which are chiefly the result of muscular exertion are normally neutralized through the agency of the suprarenal bodies.

The statement is often met with in textbooks that adrenalin acts as an antidote to muscle fatigue. Gruber (1922), Hoskins and Durrant (1923) independently have shown that massive doses of adrenalin temporarily heighten the muscular contractions and consequently increase the efficiency. At present studies are being made concerning the factors involved in the question whether the cortex or the medulla or both are responsible for the marked deviation from normal encountered in the experiments herein recorded.

CONCLUSIONS

1. A comparative ergographic study of the fatigability of the gastrocnemius muscles of adrenalectomized and normal rats was made.

2. The absolute strength per gram muscle of the experimentals was slightly higher than that of the controls.

3. The total work performed by the adrenalectomized animals before complete fatigue was only $\frac{1}{16}$ of that of their controls.

We wish to acknowledge our indebtedness to Dr. R. G. Hoskins and Dr. Milton O. Lee for advice and criticism in regard to this investigation.

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STUDIES ON VIGOR

X. The Effects of Ovarian Extirpation on Fatigability of Muscle in the Rat

HUGH H. MILEY

From the Department of Physiology, Ohio State University, Columbus, Ohio

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Hoskins (1) has shown that castration in male rats results in a decrease of about 60 per cent in their voluntary activity, as measured by the revolving cage method. Wang (2) has shown that after ovariectomy the voluntary activity of the female is decreased from 90 to 95 per cent, and further that the cyclic rhythm of activity dependent upon the oestrual cycle is abolished. These results have been confirmed by Durrant (3).

Work has been in progress in this laboratory on some of the factors concerned in this decrease in voluntary activity following castration or ovariectomy. Gans and Hoskins (4) in ergographic tests of normal and castrated male rats have shown that the total amount of work that could be done by a gastrocnemius muscle was considerably less in the castrate than in the control. They found that the weights of the individual gastrocnemius muscles were greater in the castrated than in the normal animals, but that the absolute strength per gram of muscle averaged approximately the same in each group.

This paper reports the results of similar tests in normal and spayed female rats. These results agree with those from the male series, but are even more striking.

Methods and observations. Fifty female rats, varying in ages from 30 to 95 days, were placed in revolving activity-recording cages. After a period of typing of from 14 to 45 days the best forty of these animals were divided into groups, of two or more each. The groupings were made on the basis of weight and activity records, the rats of a group being matched as nearly as possible. In general the less vigorous animals were reserved as controls, but by the time the ovariectomies were made, the activity levels had shifted so that the controls averaged somewhat higher. In some groups one animal served as control for several experiments. At ages varying from 48 to 75 days the activity showed the cyclic variations due to the establishment of the oestrual cycles. The animals designated as "experimentals" were ovariectomized following a

technique essentially the same as that described by Durrant (3). Two of the controls were traumatized and unilateral ovariectomy was performed on one animal. The animals operated upon were in most cases returned immediately to the revolving cages and left for a period of time varying from 25 to 87 days.

The average daily voluntary activity 10 days prior to ovariectomy of the experimental animals was 9,855 revolutions (29,565 feet) compared with 11,397 revolutions for the controls during the same period. Beginning at about the twentieth day, the activity of the ovariectomized animals reached a much lower level than that of the controls. This low level was maintained and activity cycles were absent throughout the remainder of the experimental period. The activity of the two series was compared over a ten-day period twenty days after ovariectomy in the one series. The activity of the controls averaged 9,898 revolutions per day, a slight increase over the initial period, whereas that of the experimental animals averaged 1,883 revolutions per day, or a decrease of 82 per cent. This difference was maintained throughout the experiment. The rhythmicity in activity of the controls did not cease and the average level of activity remained constant.

Assuming that the greater activity of the control animals was due to the presence of an ovarian hormone, the question arises: How long does this hormone persist after ovariectomy? In the cases studied of the animals completely ovariectomized the activity dropped to a low level in periods varying from 11 to 35 days. The average was 20 days and agrees with the results of Durrant (3) for four of his ovariectomized animals.

The technique used in the quantitive study of the fatigability of nerve-muscle preparation, in situ, was essentially the same as that described by Gans and Hoskins (4). The animals were anesthetized with amytal, which was injected subcutaneously so that uniform and prolonged anesthesia could be obtained. The sciatic nerve was laid bare, sectioned near the hip joint and adjusted in a modified Sherrington electrode. The skin was then sutured over the incision. A medial incision was made into the thigh: by blunt dissection the femur was exposed and fixed by means of a small modified Harvard femur clamp. The gastrocnemius muscle was isolated from its distal attachment and connected by a strong linen thread to a heavy type Harvard muscle recording lever. The approximate absolute strength of the muscle was first determined, using break shocks of a maximal faradic current as stimuli. In order to obviate fatigue this orienting study was confined to 4 trials of each muscle. An initial weight of 250 grams was attached and the nerve stimulated. Using the first reaction as a guide, lead weights of the estimated required number were either added or removed, and the stimulus repeated. In practice it was found that the fourth trial was adequate to fix the limit rather accurately.

The muscle was then stimulated automatically at one-second intervals by means of a faradic current using break shocks. The inductorium was connected to a battery of 4.2 volts and the secondary coil placed at 5 cm. The current was made and broken by a chronograph every second, the contact being maintained throughout the interval. The make stimuli were short-circuited by means of a special device illustrated in the paper referred to (4). A curved platinum connection, however, instead of copper, was used to dip into the small mercury cups. In the first few animals the muscle was after-loaded with a weight of 50 grams; for the remaining experiments an after-load of 100 grams was used.

In all, eleven groups of two, three, five or seven animals each were studied. These included 18 ovariectomized and 14 control animals. Complete data as to the work done were secured in ten groups, except for one experimental animal in a group of seven. For one control animal in a group of three the record of work done and some other data were not obtained so this group was omitted from the calculations. In twelve cases, the total work done by the control animals was greater than that by the spayed, while in one instance in a group of five an experimental animal surpassed slightly one of the two control animals. However, in this case, ovarian tissue was found at autopsy (verified histologically). This animal should possibly, therefore, be counted as a traumatized control. The ages at the time of the final observations were made ranged from 97 to 196 days. The intervals between ovariectomy and ergographic tests varied between 25 and 87 days. The work done before complete fatigue was reached was greater in the control than in the experimental animals in all cases of complete double ovariectomy. The averages for ten groups were: controls, 1,165,933 gm. cm.; experimentals, 392,066 gm. cm. Reduced to kilogram-centimeters of work per gram of muscle, the results were: controls, 751.5 kgm. cm., and experimentals, 235.4 kgm. cm. per gram (see table 1).

In eleven groups of animals activity cage records were available. These were averaged for thirty days preceding the ergographic determinations. In all instances the controls were more active than the experimentals, and in most instances the differences are striking. The averages in the eleven groups were 9,978 revolutions (29,934 feet) for the controls; 2,651 revolutions for the experimentals. The average gain in weight per week of the controls was 3.6 grams, and of the spayed animals 7.0 grams.

Animal 4044 E., in which ovarian tissue was found at autopsy, brings up the average of the experimentals. However, it should not be considered the best experimental animal. This animal's daily activity was 7,245 revolutions and the work performed was 687,940 gm. cm., thus surpassing one of the controls which did 646,340 gm. cm. of work. In spite of this the advantage of the controls is a very marked one. The

TABLE 1

Showing results of ergographic experiments

ANIMAL NUMBER	AGE	INTERVAL AFTER OVARIECTOMY	GAIN IN WEIGHT PER WEEK	WEIGHT OF ANIMAL	WEIGHT OF MUSCLE	ABSOLUTE STRENGTH	ABSOLUTE STRENGTH PER GRAM OF MUSCLE	WORK PER GRAM	WORK	ACTIVITY				DAILY ACTIVITY REVOLUTIONS
										A	B	D	F	
	days	*days*	*gms.*	*gms.*	*gms.*	*gms.*		*gm. cm.*	*kgm. cm.*					
3273E	114	57	3.9	184	0.95	120	126	59,235	62.3	2,992	1,075	0.64		1,262
2020C	114		1.7	196	1.00	200	200	174,990	174.9	3,796	2,879	0.24		2,274
3496E	160	59	2.6	234	1.50	140	94	45,382	30.2	3,382	879	0.74		915
3035C	160		4.0	233	1.86	190	106	183,456	98.6	10,487	8,843	0.15		8,910
3227E	130	65	6.3	203	1.33	130	100	135,607	101.9	5,529	2,065	0.62		1,891
†3215C	130		3.2	219	1.46	280	192	1,621,418	1,158.1	6,944	5,252	0.24		4,371
3213E	135	74	2.0	257	1.73	360	208	616,882	356.5	7,201	1,864	0.74		1,443
3211C	142		12.4	282	1.43	410	286	1,347,053	962.1	3,854	2,986	0.22		3,666
3231E	140	81	2.0	280	1.75	230	131	864,940	494.2	11,642	1,883	0.83		3,653
S3233C	128		1.8	243	1.60	280	175	1,398,250	873.9	6,293	7,805		0.19	9,646
3296E	138	58	7.2	236	1.20	170	141	146,374	121.9	3,260	1,211	0.62		1,975
3235C	124		0.9	198	1.73	260	150	386,942	223.6	11,056	9,845	0.1		9,514
T4044E	186	84	8.6	235	1.9	280	147	687,940	362.0					7,245
4014E	196	84	4.5	205	1.53	170	111	308,610	201.7	9,496	656	0.93		1,484
4049E	175	87	5.3	230	1.67	210	125	384,940	230.4	16,746	855	0.94		1,173
4035C	181		1.9	193	1.53	340	222	646,340	430.9	14,337	11,925	0.16		13,438
T4043C	177		7.9	226	1.83	350	191	2,205,671	1,225.3	15,263	13,748	0.09		11,786
3217E	194	77	4.5	245	2.03	170	83	5,347	26.3	5,030	2,641	0.47		3,358
3212E	178	61	7.3	284	1.46	240	164			9,390	4,219	0.55		3,949
2149E	178	59	9.1	254	1.70	420	247	587,490	345.5	14,271	2,446	0.82		2,710
4024E	148	40	8.7	192	1.48	170	114	893,600	603.0	28,677	524	0.98		683
3293C	170		5.1	252	1.60	380	237	1,843,570	1,152.2	18,380	14,693	0.2		15,169
2870C	130		1.8	200	1.54	330	214	1,355,800	903.8	14,887	15,035		0.009	13,684
S4055C	126		1.5	161	1.30	250	192	968,450	744.9	15,923	12,393	0.22		13,916
4077E	124	50	6.6	193	1.50	290	193	348,680	232.4	8,711	1,562	0.82		2,612
2644E	155	40	9.2	260	1.85	330	178	647,454	349.8	8,434	1,778	0.78		1,980
2570C	97			144						18,542	16,340	0.11		15,120
R2316E	160	25	17.1	276	2.13	200	93	555,821	260.9					1,555
R2633E	148	26	10.8	237	1.83	300	163	320,537	176.2					2,579
2044C	130		3.8	214	1.42	220	155	1,006,369	718.8	11,038	9,687	0.12		8,110
4023E	194	87	11.7	305	3.30	410	124	156,290	47.3	13,076	4,590	0.64		7,256
2047C	160		0.9	188	1.33	240	179	2,663,900	2,049.1	8,765	7,146	0.18		10,091
*93C	131			176	1.4	600	428							
*2569C	120			145	1.23	290	241	867,796	723.1					
*25C	137			220	1.40	690	492	819,000	585.0					
Averages:														
Controls	141		3.6	205	1.39	331	238	1,165,933	751.5	11,397	9,898	0.131	0.099	9,978
Experimentals	159		7.0	239	1.71	241	141	392,066	235.4	9,855	1,883	0.82		2,651

* Excluded from averages except absolute strength.

† Unilateral ovariectomy.

C, control animals; E, experimental animals; T, ovarian tissue found upon histological examination (T, originally E); S, sham operation (laparotomy); R, in revolving wheels too short a period to include activity before and after ovariectomy and per cent drop in activity with the rest. A, activity of C and E (average for ten days previous to the time of ovariectomizing the experimentals); B, activity of C and E (average for ten days following a period of twenty days after ovariectomizing the experimentals); D, per cent drop in activity of C and E; F, per cent gain in activity of C.

results obtained agree with other experiences in this laboratory and elsewhere that sham laparotomies have little or no permanent effect on the activity of the animals.

The results of the ergographic experiments correspond with the observed greater general level of activity of the females over that of the males and also with the greater decrease of activity following ovarian removal over the decrease following castration. In various investigations it has been noted that the general level of activity of males is lower than that of females. Hitchcock (5) reported a series in which the activity of males was only 56 per cent of that of the females. Wang, Richter and Guttmacher (6) state that the average daily activity of the male varies between two and eight thousand revolutions while that of the female varies between six and twelve thousand. The females used in these experiments did considerably more work than the males used in the experiments reported by Gans and Hoskins (4). The results are not exactly comparable, however, because of the slight modifications of the technic. However, it can be definitely stated that gram per gram muscle from female rats is able to do more work than that from males.

In view of the well-marked difference in efficiency the question of weight and absolute strength of the muscle tested is of primary interest. The weights of the individual muscles were greater in the ovariectomized animals but the absolute strength per gram of muscle was greater for the controls. In the males the absolute strength per gram of muscle averaged approximately the same in each group. In the females there is an increase of 23 per cent in the average weight of the muscle of the experimental animals as contrasted with that of the controls, while a comparison of the absolute strength per gram shows a decrease of 40 per cent in the experimentals.

The total activity of the different animals throughout the time of this experiment varied within fairly wide limits. The most active rat in the control series, no. 4035, made a total of 1,800,820 revolutions for a period of 134 days, or an average of 13,439 revolutions per day. Of the experimental animals, no. 4049 made 787,380 revolutions for the same length of time, or an average of 5,875 per day. The least active control rat made 147,800 revolutions for a period of 65 days, or 2,274 revolutions per day and the least active experimental, 106,380 revolutions in 67 days or 1,636 per day. A comparison of the ergographic and revolving cage records of the best and poorest rats shows: the best control, no. 2047, did 2,663,900 gm. cm. of work correlated with a daily activity of 10,091 revolutions; the poorest control, no. 2020, did 174,990 gm. cm. of work and showed a daily activity of 2,274 revolutions. The best experimental, no. 4024, did 893,600 gm. cm. of work and had a daily activity record of 683 revolutions; the poorest experimental, no. 3217, did 5,347 gm. cm. of work and had a daily

activity of 3,358 revolutions. These data are summarized graphically in figure 1.

Speculations as to underlying reasons for the depression observed in spayed animals would seem at this point rather unprofitable. However, the greater amount of work performed by the normal females, the absence of oestruation in ovariectomized animals and its presence in normal mature females indicate that the ovaries are at least secondarily involved.

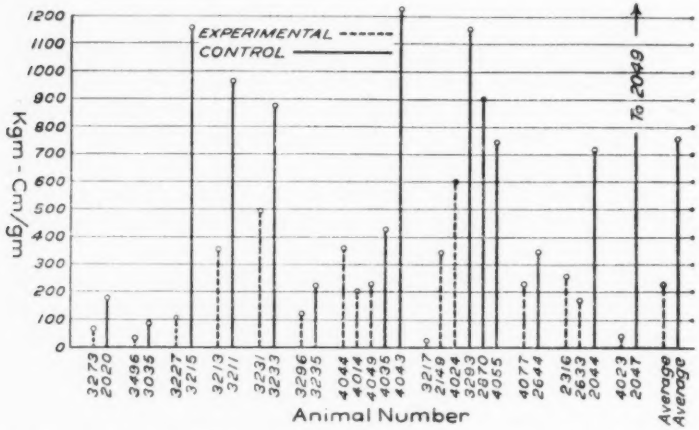

Fig. 1. Work done by gastrocnemius muscles of ovariectomized and normal rats. The muscles were stimulated through their motor nerves to the point of complete fatigue. They were afterloaded with 100 grams' weight.

SUMMARY

1. A comparative study was made of the absolute strength and fatigability of the gastrocnemius muscles of ovariectomized and normal female rats. The muscles, in situ, were stimulated through their motor nerves.

2. Ovariectomized animals gained weight more readily than their controls and the weights of the individual muscles were greater in the spayed animals. The absolute strength per gram of muscle averaged 40 per cent greater for the controls than for the experimentals.

3. The proportion of gastrocnemius weight to total body weight average the same in each group.

4. The total work performed by the ovariectomized animals was only 33.6 per cent of that done by the controls. This agrees well with the decrease in voluntary activity following spaying.

5. The average daily activity of the castrated males in Gans and Hos-

kins' (4) experiments was 2,298 revolutions while that for the spayed females in these experiments was 2,651 revolutions.

This problem was suggested by Dr. R. G. Hoskins and carried on under the direction of Dr. Milton O. Lee. I wish to acknowledge my indebtedness to them for advice, criticism and for other kinds of help. I am also indebted to Mr. Howard M. Gans for technical assistance.

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STUDIES ON VIGOR

XI. Relation of Hysterectomy to Voluntary Activity in the White Rat

E. P. DURRANT

From the Department of Physiology, The Ohio State University

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Both from clinical observations and from anatomical considerations there have for some years been notable differences of opinion as to the physiological importance of the uterus other than as an organ of gestation and as the immediate seat of menstrual activity. Thus some have held that total ablation of the uterus is ultimately followed by atrophy of the ovary as a result of a lack of the internal secretion normally supplied by the uterine glandular tissue. Among those holding this view are Loewenthal (1), Zweifel (2), Abel (3), Engström (4), Doran (5) and Graves (6). Others, among them Selheim (7), Holzbach (8), Bond (9) and Carmichael and Marshall (10) incline, on the contrary, to the view that the ovaries are not functionally dependent upon the uterus.

The earlier reports are based on collection and analysis of clinical records of operations performed primarily for the relief of pathological conditions, and obviously are not ideal as research material. Bond, from experiments on a few rabbits, and Carmichael and Marshall, from a systematic study of a considerable number, found that the removal either of the entire uterus or of all but the cervix had no effect on ovarian development in immature animals. The latter workers also performed hysterectomy on full-grown rats, the subsequent examination of which showed no degeneration of the ovaries after several months.

In experiments on the guinea pig, Loeb (11) has shown a prolongation of the life and function of the corpus luteum following the removal of all or a part of the uterus, with the indirect effect of preventing ovulation as long as the corpus functions. On the other hand, Hartman (12) has reported that hysterectomy has no effect on the oestrous cycle in the opossum. Long and Evans (13) make a similar report in regard to the white rat. In view of this lack of agreement both among the deductions from clinical data and those from animal experimentation it seemed desirable to extend our knowledge of the question, if possible, by means of further studies on the white rat, whose cyclic ovarian activities can be

followed not only by the vaginal smear method described by Long and Evans, but also by the periodic variations in voluntary activity described by Wang (14) and Slonaker (15).

METHODS. A series of twelve animals was first typed by the vaginal smear method, daily observations being made and recorded until the length of the oestrous cycle could be determined. This required on the average 32 days. The uteri were then removed as follows: after application of mercurochrome solution over the field of operation, a mid-ventral incision 12 to 15 mm. long was made ventral to the urinary bladder; the uterus was drawn up and a strong cotton thread tied round the cervix. Without further ligation the cornua were excised by section just distal

TABLE 1

First series

RAT NUMBER	AGE AT OPERATION	DAYS CYCLE SUSPENDED	AVERAGE LENGTH OF CYCLE	
			Before hysterectomy	After hysterectomy
954		24	6.0	5.0
982	122	22	5.0	4.6
991	122	20	5.3	4.8
994	126	23	5.3	5.0
995	126	13	4.2	4.6
996	126	20	4.6	4.2
997	126	20	4.0	4.6
998	121	24	4.7	4.3
1001	108	28	4.3	4.6
1002	108	26	5.4	5.0
1003	110	33	5.3	5.4
684		13	*	4.0
Average			4.9	4.7

* No record before hysterectomy.

to this ligature and through the oviducts. The peritoneal cavity and the skin incision were closed separately by silk ligatures. A collodion and gauze dressing was also applied. Ether was used for anesthesia. The animals were all returned to their usual quarters within an hour after the operation. There were no fatalities, although one of the controls had to be destroyed ten days before the conclusion of the experiment on account of middle ear infection. In this series the ten whose ages were known were from 108 to 126 days old at the time of the operation. The others were approximately the same age. The second series consisting of twenty animals was observed primarily by means of activity cages substantially as used originally by Stewart (16), but with some slight modifications

made in this laboratory, as elsewhere described (17). This series was first placed in the revolving cages and daily readings of the number of revolutions kept for thirty days. By plotting the daily activity the cyclic increases corresponding to oestrus, as described by Slonaker and by Wang, were clearly shown. At the end of thirty days of typing in this manner, 14 animals were selected for hysterectomy so as to leave litter controls for most of them. The operation was carried out in the same manner as in the first series and the animals at once returned to the cages. The activity observations were supplemented near the close of the experiments by vaginal examinations.

TABLE 2

Second series. Daily average number of revolutions of 14 hysterectomized (H.) and 6 control (C.) white rats for: A, 20 days preceding; B, 5 days succeeding operation; C, 20 days succeeding period B

RAT NUMBER	DAILY AVERAGE NUMBER OF REVOLUTIONS			RAT NUMBER	DAILY AVERAGE NUMBER OF REVOLUTIONS		
	A	B	C		A	B	C
600 C.	4,200	3,700	3,100	1032 H.	16,000	10,600	18,900
614 H.	2,100	1,900	1,700	1033 H.	17,200	6,500	10,700
1009 H.	6,800	2,500	4,400	1034 H.	12,600	1,700	11,900
1010 C.	15,700	19,200	9,800	2024 H.	11,600	6,400	10,600
1011 H.	15,400	9,500	10,700	2026 H.	10,300	3,300	9,700
1012 H.	16,800	9,100	15,000	2028 C.	12,500	10,200	11,500
1015 C.	14,000	12,600	14,500	2064 H.	18,000	6,400	13,500
1016 H.	19,000	11,800	17,700	2065 H.	10,600	5,000	8,800
1019 H.	17,900	9,100	15,800	2066 H.	13,400	2,800	7,400
1031 C.	17,500	14,100	13,900	2067 C.	8,900	7,400	9,100
Average of six controls					12,100	11,200	10,100
Average of fourteen hysterectomy					13,500	6,400	11,600

RESULTS. The effect of hysterectomy in the first series was the disappearance of the cyclic vaginal changes within three or four days after the operation. In from 13 to 33 days the oestrous cycle was completely restored, the average time being 23 days. These data are given in detail in table 1, together with the average length of the cycle before and after the operation. In the second series the effect of hysterectomy was, as shown on the accompanying graph which is fairly typical of the series as a whole, an immediate fall in activity followed by a large increase at the next cycle and complete recovery at the succeeding one (fig. 1). In the twenty days following the operation the average daily activity of the experimental group fell off 16.5 per cent and that of the controls 17.0 per cent (table 2). The oestrual rhythm was practically unchanged.

Discussion. The temporary cessation of the cyclic vaginal changes following the extirpation of the uterus in this experiment were doubtless due to operative interference with the vaginal circulation, similar results being reported by Long and Evans, though the recovery was somewhat quicker in their animals. The rhythmic development of ova apparently was not disturbed, the collateral circulation through the ovaries being sufficient to meet ordinary nutritional requirements. Thus also the rhythm of voluntary bodily activity, shown by Wang to be correlated with the oestrous cycle whose manifestations are explained by Allen and Doisy (18) as due to the action of a hormone produced in the Graafian follicle, is practically unaffected by the operation of hysterectomy. From numerous observations of traumatized controls in this laboratory, it is evident that the marked decrease in total activity immediately subsequent to the removal of the uteri was due to the operation per se. Hartman, in dis-

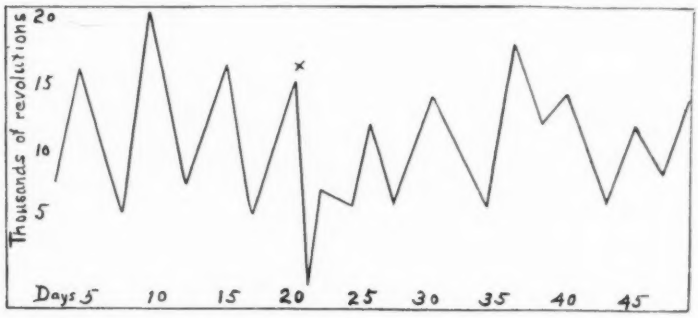

Fig. 1. Graph of cyclic voluntary activity of female white rat showing oestrual increases. At *X* hysterectomy was performed.

cussing similar results in the opossum suggests that the slight remaining stumps of the cervix might be sufficient to stimulate the ovaries to maintenance of their normal rhythm, but the work of Long and Evans showed that neither the removal of the uteri nor the complete excision of the cervix had any effect on the oestrous rhythm. In regard to clinical observations, Abel quotes Engström, with whom he agrees, as follows: "Eine misgestaltete und nicht ganz functionirende Gebärmutter ist besser als gar keine." This may be true for psychological reasons but is probably worthless as far as the preservation of any sources of possible hormone influence on the ovary is concerned. The experimental and clinical evidence given by Selheim, Bond, Holzbach and others is abundant and positive to the effect that the uterus is without influence on the functional activity of the ovaries. The last author says that as a rule the ovaries do not atrophy after hysterectomy, and when they do it is probably due to surgical interference with nervous connections.

SUMMARY

In an experiment with 26 hysterectomized and 6 control white rats observations were made by means of vaginal smears and by recording voluntary activity. The results indicate that the uterus exerts no hormonal effect on the cyclic activity of the ovary. Our observations carried out by the revolving cage confirm the results of the work of Long and Evans by the vaginal smear method.

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THE FAILURE OF HISTAMINE TO INDUCE OESTROUS CHANGES IN SPAYED RATS

PAUL M. LEVIN

From the Anatomical Laboratory, School of Medicine and Dentistry, University of Rochester

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Allen and his co-workers in 1923–1924 reported the detection of oestrous changes in spayed rats, following the injection of an extract of pig's follicular fluid. Similar active extracts have been obtained from the corpus luteum, placenta and the circulating blood of various mammals, and from certain of the lower vertebrate reproductive tissues. The fatty residues of these extractions, similar in their behavior, have been taken to be of a specific nature, and have been called, for example, the "female sex hormone" (Frank).

Attempts to test this specificity by the trial of other substances have practically all brought concordant results—in almost every experiment the substituted material failed to induce oestrous changes. Extracts of the pituitary, adrenal, liver, spleen and brain have consistently yielded no change in the reproductive tracts of the experimental animals (Zondek and Aschheim, 1926; Frank *et al.*, 1926; Robinson and Zondek, 1924). The last-named authors, using the mass of the excised uterus as a criterion for the effect of injected substances, described uterine growth in some cases following the injection of testicular, thymic and pineal extracts. But especially they found that histamine, as well as some other nitrogenous substances, produces hyperemia, increase in thickness and enlargement of the guinea pig uterus. However, Robinson and Zondek did not mention any operative castration of their animals, so one may assume that they dealt with guinea pigs that had their ovaries intact. It follows, of course, that any positive results obtained are open to doubt as to their significance in the causation of oestrus.

The use of histamine is reported in one other paper, that of Frank and his collaborators (*loc. cit.*). These authors used immature rabbits and spayed rats, and found that histamine induces no growth in the uterus or oestrous changes in the vaginal contents.

In view of the discordance in the results of histamine injections, and of the important physiological reactions of this substance, it seemed desirable to test further the effects of the administration of histamine on the reproductive tract of the rat.

Four mature female albino rats were used in this work. The oestrous behavior of these rats was found, by the vaginal smear method, to be normal. Bilateral oöphorectomy was performed on each rat, and in all, cyclic variations in the vaginal contents ceased. Vaginal smears were made at least every 24 hours, and in special cases, at intervals as frequent as six hours.

First, observations of the effect of the oestrus-producing principle were made with the use of material from two sources. The first was an extract of pig's follicular fluid, prepared by the author according to the method of Ralls, Jordan and Doisy (1926). The second was the substance "Estrogen," an extract of human placenta prepared by Parke, Davis & Co. Both of these extracts, when injected subcutaneously in adequate amounts, were found active. The vaginal contents of the injected rats showed a complete series of oestrous changes including the disappearance of the leucocytes, the appearance of large numbers of cornified cells, and finally the reappearance of leucocytes.

With this background, experiments with histamine were begun on the same rats, five days after the positive action of the two above-mentioned extracts. The histamine used was "Ergamine acid phosphate," prepared by Burroughs Wellcome Co. Injections were made subcutaneously, to permit gradual absorption of the drug, and the total dose was given in several fractions over a period of about six hours. The lethal dose, when injected in aqueous solution subcutaneously into spayed rats, was determined to be about 0.32 gram. (This dose produced the state of histamine shock five hours after the injection of the first portion; death followed in about six hours.) The quantities administered to each rat were as follows: To rat 1, 0.05 and 0.25 gram; to rat 2, 0.10 gram; to rat 3, 0.32 gram; to rat 4, 0.15 and 0.20 gram. (There were six experiments in all.)

It should be noted that the criterion for oestrus that was used was the disappearance of leucocytes from the vaginal secretion, not the appearance of cornified epithelial cells. The reason for this is that the latter type of cell practically always appears in varying numbers in the normal "dioestrous" smears of spayed rats. Periodic fluctuations in the proportions of cornified cells were not regular; no cycles could be made out.

In no case did the signs of oestrus appear in the vaginal contents of the rats injected with histamine. Indeed the only effect of the histamine injections that was observed was a possible slight increase in fluidity of the vaginal secretion. Four days after the last injections of histamine, the rats were again injected with "Estrogen," and all gave a positive vaginal reaction; this control injection demonstrates that the negative results of histamine-injection were not due to any degenerative change in the rats.

These experiments, therefore, fully corroborate those of Frank. The use of histamine offers us nothing positive concerning the action of the oestrus-inducing principle.

The writer expresses his thanks to Prof. George W. Corner for suggestions and advice.

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ON THE EXISTENCE OF A PARATHYROID HORMONE

FREDERIC T. JUNG

From the Hull Physiological Laboratory, University of Chicago

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That the parathyroid glands are able to perform their function satisfactorily after removal from their normal location to other parts of the body has been adequately shown by the transplantation experiments of Erdheim (1911) and Biedl (1913). This leads to the conclusion that they act chemically upon materials brought to them by the blood and that the products of their action are carried away by the blood or lymph. It is obvious that the disappearance of one group of substances and the appearance of a second group in the fluids passing through a gland can be merely the two aspects of a single characteristic chemical reaction which in this case is one that cannot apparently go on with equal facility in any other tissue in the body. The consequences of extirpating the parathyroids must necessarily be *both* an accumulation of materials which they have hitherto removed and an insufficiency of the products which they have hitherto elaborated. If there exist in the body no other mechanisms for preventing the accumulation of such materials, and if these materials be detrimental, then parathyroidectomy will be followed by an intoxication. If, on the other hand, there exist no other mechanisms for elaborating the characteristic parathyroid product, parathyroidectomy will be followed by deficiency symptoms. In the former case the glands would be said to act by detoxication; in the latter case they would be said to act by producing an autacoid.

The preparation of active extracts of parathyroid tissue by Vines (1923), Hanson (1923) and Collip (1925) is a cogent item of evidence in favor of the hormone or autacoid theory. To the work with these extracts there is the objection that strong reagents are used in their preparation, so that the product may contain chemical artifacts which might only accidentally simulate certain phases of parathyroid action and which might be said to act pharmacologically rather than physiologically. The experiment to be described here was done in an effort to meet this objection.

It was suggested by Doctor Carlson that the parenteral administration of dead parathyroid tissue to parathyroidectomized animals might avoid the objections against extraction methods and still be able to reveal a store of active substance in the gland. The difficulties of this experiment

would be, first of all, to find a method of insuring the death of the tissues without subjecting them to powerful physical or chemical agencies, and second, to find a satisfactory test for the activity of the administered tissues in case their hormone content, if any, were low. The former difficulty might be met in this way: the parathyroid tissue from a given animal might be given to another of different species by an implantation method, the difference in the species chosen being such as to prevent a possible "taking" of the graft. The tissue so removed would undergo asphyxia, necrosis and absorption, and would thus liberate its store of hormone. The second difficulty would have to be met by using a sufficiently large number of animals, so that even if the implantation was not potent enough to save every animal, its prophylactic effect would be manifested in a general or statistical tendency.

A number of preliminary experiments by Mr. F. D. Harper soon showed that cat or dog parathyroids implanted in a rat after parathyroidectomy could by no means be depended on to save the rat. Especially in young rats the progress of the parathyroprival symptoms is so rapid that apparently the absorption of the foreign parathyroid was too slow to be effectual.

Procedure. In the experiment here reported, therefore, the implantations were done first, so as to give the absorption process time to get under way. Three groups of animals were used.

A. Group A was a number of rats in which nothing but thyroparathyroidectomy was done. They are referred to as the "untreated" series, and must not be confused with the true controls.

B. Group B consisted of the test animals. The history of any rat in this series was as follows: (1) Implantation (aseptic, under ether anesthesia) of cat or dog parathyroids, (2) an interval of one or two days, (3) thyroparathyroidectomy, (4) observation of subsequent course of rat.

C. Group C consisted of the controls. They first received an implantation of spleen, salivary gland, or testis from cat or dog; then, after an interval of a day or two, they were thyroparathyroidectomized. They therefore underwent two etherizations, just as the test animals did, and the bits of tissue implanted in the controls were of the same size as the parathyroids used in the test animals.

Results. A revision of the data was necessary in order to exclude rats in which necropsy showed the extirpation to have been incomplete or in which various other factors had operated in such a way as to cause "selection." This considerably reduced the numbers finally compared. The results are given in table 1.

The interpretation of these data was not easy. In many respects the untreated rats suffered less than either of the other groups. Ignoring this group (since it was pointed out above that they were not the true controls), we compare groups B and C, and find at first sight a perfect unanim-

ity of evidence proving that the parathyroid implants operated in favor of the rats of group B. However, this conclusion could not be accepted without further investigation. In the first place, the difference between B and C is not so great as to preclude the difficult question whether the difference was really outside the range of experimental error. Further, it must be pointed out that items (a) to (e) cannot be regarded as so many independent corroborations of each other, for it is evident that the inclusion of a single incompletely parathyroidectomized rat in, say, group B would simultaneously affect all of the data under B in the table.

To test the conclusion more carefully, the two sexes were studied separately in each series; it was found that with only two trivial exceptions the

TABLE 1

	UNTREATED A	TEST B	CONTROL C
Number of rats represented	27	29	14
a. Time of onset of tetany (median value for each series) in hours	77	41	18
b. Percent of rats showing tetany	56	59	86
c. Severity of tetany (in arbitrary units, 3 representing violent tetany and 0 representing absence of tetany)	1.24	1.03	1.64
d. Minimum longevity (hours)*	113.7	259.8	245.8
e. Maximum longevity (hours)*	119.3	283.0	251.3
f. Percent of survivals over 25 hours	74	93	64
f. Percent of survivals over 50 hours	48	83	57
f. Percent of survivals over 75 hours	30	66	57
f. Percent of survivals over 100 hours	26	62	50
f. Percent of survivals over 125 hours	15	59	50

* For each rat, in general, two numbers representing length of life after operation were obtained: the number of hours from operation to time when last seen alive, and the number of hours from operation to time when found dead. When the death of the animal was actually observed, the numbers were, of course, equal.

data for each sex separately showed the same differences among the three groups as are seen in the above table. Finally, however, a striking fact emerged when the mortality data (f) were put into graphic form.

It is seen that the untreated rats of group A give the smooth mortality curve which one would expect. The shapes of the curves for groups B and C, however, strongly suggest the following *hypotheses:*

1. The rapid initial death rate in group C is due to the detrimental effect of the decomposing foreign tissue in the body of the rat. The absorption of this tissue manifests itself in the curve up to about the 75th hour. After that a different factor operates in favor of the rats in this group, the fact that, of the rats which had been intended for this group, those most sus-

ceptible to the dangers of etherization and infection have been selected out by a preceding (implantation) operation, so that group C consists of more resistant rats than does group A.

2. The surprising initial convexity of the curve for group B is due to a protective effect of the parathyroid implant. This effect is visible up to

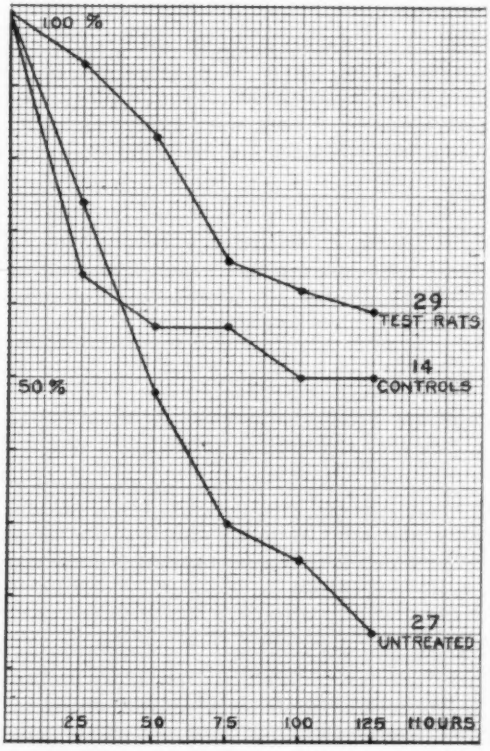

Fig. 1. Graphic representation of data (f) of table 1. The ordinates represent the percentage of rats surviving thyroparathyroidectomy by the number of hours indicated by the corresponding abscissae.

the 75th hour. After that the same factor noted above (the fact that the less resistant individuals have been selected out by a previous operation, leaving group B composed of more resistant rats than group A) operates in favor of group B.

It is readily seen that if the controls had not been properly planned, an exaggerated idea of the value of parathyroid implants would have resulted.

Nevertheless, careful study of the data leads definitely to the conclusion that the parathyroid implants protected the rats.

Discussion. The protective effect of the parathyroid implants could be brought about in two ways: 1, by a brief survival of the tissue, which could thus resume for a time its supposed detoxicating function, and 2, by absorption of the implanted tissue, which would thus liberate into the rat its store of autacoid.

The first possibility was eliminated by the precautions which were taken to prevent survival of the tissue. The tissues to be implanted were always kept for at least several hours in sterile 0.9 per cent NaCl in an ice-box before implantation. They were looked for in every necropsy. They usually disappeared within a few days; the longest persistence ever seen was 35 days in the case of a rather large piece of spleen, and this piece was found on microscopic examination to be completely hyalinized. In view of all these facts, a temporary resumption of activity by the implants, in foreign body fluids, seems highly improbable. The evidence is therefore strongly in favor of the liberation of a stored-up autacoid by the implant as it underwent absorption.

SUMMARY

When the parathyroid glands of cats and dogs are implanted into parathyroidectomized rats, the deficiency symptoms in the rats are slightly but definitely mitigated. The experiment having been done under conditions which made survival and functioning of the implant in the recipient extremely unlikely, one must conclude that the parathyroids contain a store of a hormone which is not species-specific, nor a chemical artifact, nor contained in the control tissues used.

The author gratefully acknowledges his indebtedness to Dr. A. J. Carlson for proposing the problem and for much help and advice. He also wishes to record his appreciation of the assistance of Mr. Earl O. Latimer in nearly all of the operations.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE PANCREAS

III. A Hormone for External Pancreatic Secretion[1]

A. C. IVY, J. I. FARRELL AND H. C. LUETH

From the Department of Physiology and Pharmacology, Northwestern University Medical School

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The literature bearing on this problem has been reviewed in the preceding article (1926) of this series, in which a humoral mechanism for external pancreatic secretion was proved. Attempts to throw light on the nature of the humoral agent, or agents, suggested that it is a hormone.

If it were possible to devise experiments in which the possible action of secretagogues could be avoided, positive proof of a hormone would result. In this paper we will report the results of experiments devised for this purpose.

Methods. *Thiry fistula and pancreatic transplant preparation.* A Thiry fistula of the jejunum was made in a dog that had a pancreatic transplant (Ivy and Farrell, 1926). The mucosa of the Thiry fistula could be washed, kept free from food, and substances applied to it at will.

Jejunal and pancreatic transplant preparation. A twelve-inch loop of jejunum was transplanted under the skin and at a later operation a pancreatic transplant was made.

The operation was performed as follows: *First stage.* A dog with hypertrophied mammary glands was selected. Through a mid-line incision beginning at the umbilicus a twelve-inch loop of jejunum was resected with pedicle intact, the integrity of the bowel being reëstablished by an end-to-end anastomosis. A bed was prepared on both sides just lateral to the incision in the tissue beneath the mammary gland. A one-inch skin incision was made above and below each mammary gland and was connected with the prepared bed by blunt dissection. The abdominal incision was closed, beginning at the lower end, up to within three-fourths of an inch from the upper end, leaving an opening in the abdomen through which the pedicle of the loop passed. The loop was then divided and the ends of each half brought to the outside through the incision above and

[1] Demonstration and preliminary report. Proc. Soc. Exp. Biol. Med., 1926, xxiii, 753; Trans. XIIth. Internat. Cong. Physiol., Stockholm, August, 1926, Skand. Arch. für Physiol., xlix, 1926.

below each mammary gland, each end being held in place by four sutures. The remaining layers of the abdominal incision were closed. *Second stage.* After several weeks had passed a pancreatic transplant was made at which time the pedicle of the transplanted loop was tied and severed. (See fig. 1.)

The intestinal transplant has been done in two dogs. In the first dog the loop was not divided, but was placed in the subcutaneous tissue so that it formed a "V." After the pedicle was sectioned a perforation was accidentally produced at the angle of the loop, which resulted in an abscess. As a result only four inches of the loop were preserved. This was a sufficient amount of intestine, however, to yield positive results in certain experiments.

Fig. 1. This is a photograph of one of the dogs with a jejunal-loop transplant and a pancreatic transplant. The elevations of the skin caused by the loops and the fistulae of the loops are plainly visible. The arrow points to the opening of the duct of the transplant.

RESULTS. OBSERVATIONS ON "THIRY-FISTULA-PANCREATIC-TRANSPLANT" DOGS. *The application of water.* Since we desired to determine whether water might cause either the absorption of secretagogues formed by autolysis of cellular detritus in the lumen of the Thiry fistula, or the formation of a hormone by the intestinal mucosa, we perfused without pressure the Thiry fistula with 150 cc. of tap, or distilled, water at body temperature for one-half an hour. This procedure always failed to modify the continuous secretion of the pancreatic transplant.

If the water did cause some absorption of secretagogues, or formation of a hormone, it was insufficient to excite the transplant.

The application of hydrochloric acid. In these experiments we washed out the Thiry fistula with warm water for a period of approximately three minutes, i.e., until the water returned was clear, before we applied the

acid solutions. This was done to remove secretagogues as far as possible, so that if the acid solutions on application caused stimulation, it would not be very likely that the stimulation would be due to the acid facilitating the absorption of secretagogues preformed in the intestine by autolysis of cellular detritus.

Our results show that the application of hydrochloric acid from N/10 to N/120 to the Thirty fistula by perfusion under no pressure stimulates the pancreatic transplant to secrete after a latent period of from four to six minutes. Gelatin solutions[2] with a pH of 4.0 and 5.0 when applied do not cause stimulation. Typical results of hydrochloric acid stimulation are shown in table 1.

The application of hydrochloric acid after ligation of the common bile duct. Since Ivy and McIlvain (1923) have shown that the application of acid

TABLE 1

Response of pancreatic transplant on the application of acid to a Thiry fistula of the jejunum

Dog. "Shep"

FROM	TO	TIME	PROCEDURE	AMOUNT	REMARKS
		hours		*cc.*	
5:30	7:30	2	Control, continuous secretion	0.30	
7:30	8:00	½	Applied N 10 HCl for 10 minutes	2.00	Latent period 4 minutes
8:00	11:00	3	Control, continuous secretion	0.91	
11:30	12:00	½	Control, continuous secretion	0.28	
12:00	1:00	1	Control, continuous secretion	0.25	
1:00	1:30	½	Applied N 120 HCl for 30 minutes	0.85	
1:30	2:30	1	"Recovery"	0.42	

to a Thiry fistula promotes the flow of bile and even leads to the regurgitation of rather large quantities of bile into the stomach, it is necessary to show that the stimulation due to the application of acid to the Thiry fistula is not due to bile stimulation. This possibility is important because we know from our own observations, and those of Mellanby (1926), that bile stimulates pancreatic secretion when introduced into the intestine.

In order to answer this question we studied the response of the pancreatic transplant to acid application in Thiry fistula dogs before and after double ligation of the common bile duct.

Our results (example in table 2) prove that the stimulation of the pancreatic transplant by application of acid to the Thiry fistula is not due to bile flowing into the duodenum from the biliary passages.

[2] We thank Dr. C. J. Farmer for the gelatin solutions.

The application of acid after the injection of atropine. Because the application of acid to a Thiry fistula of the jejunum stimulates gastric secretion, and also because atropine does not abolish secretin stimulation of the pancreas, it is important to determine whether the stimulation of the transplant occurs after the injection of atropine.

For this work the dogs in the preceding experiments after common bile duct ligation were used. From 10 to 30 minutes after the injection of

TABLE 2

Showing the response of the pancreatic transplant to acid application after the injection of atropine and ligation of the common bile duct

FROM	TO	TIME	PROCEDURE	AMOUNT	REMARKS
		hours		*cc.*	
11:00	11:30	½	Control	0.42	Dog 2
11:30	12:00	½	Control	0.43	
12:00	12:30	½	Control	0.43	
12:30	1:00	½	Perfused with N/10 H 1.5 mgm. Atropine injected at 12:20	0.76	
1:00	2:00	1	"Recovery"	0.81	
9:30	10:00	½	Control	0.56	Dog 2
10:00	10:30	½	Control	0.50	
10:30	11:00	½	Common bile duct ligated 5 days before this expt. Perfused with N/10 HCl	0.68	
11:00	12:00	1	"Recovery"	1.31	
12:00	1.15	1¼	"Recovery"	2.26	
10:45	12:20	1½	Control	0.89	Dog 1
12:20	12:50	½	1.5 mgm. atropine injected at 12:10. Perfused with N/10 HCl	0.74	
12:50	2:00	1	"Recovery"	0.31	
11:00	12:00	1	Control	0.22	Dog 1
12:00	1:00	1	Control	0.20	
1:00	1:30	½	Common bile duct ligated 6 days before this experiment. Perfused with N/10 HCl	0.42	
1:30	2:30	1	"Recovery"	0.20	

1.5 mgm. of atropine sulphate subcutaneously, the acid (N/10 — N/20 HCl) was applied.

Our results (example in table 2) show that the application of acid to the Thiry fistula of a dog with the common bile duct doubly ligated stimulates the transplant even after the injection of atropine in sufficient doses to abolish gastric secretion. The atropine in some experiments with N/20 HCl prevented stimulation.

These results have a threefold significance in that they show that the stimulation of the transplant caused by the application of acid to the

Thiry fistula of the jejunum is 1, not due to bile flowing into the duodenum; 2, is not due to gastric juice flowing into the duodenum, and 3, acts somewhat similarly to secretin in the atropinized animal.

OBSERVATIONS ON JEJUNAL AND PANCREATIC TRANSPLANT DOGS. Two of these animals were prepared according to the method described above and were used for experiments.

It is interesting to note that the intestinal transplant secretes normal intestinal juice; that it shows periods of peristaltic activity, mechanical stimulation augmenting the activity; that the peristalses move in both directions, but most frequently move caudally; and that it shortens and elongates. These movements can be seen perfectly as the transplant is just beneath the skin. We now have one of these preparations which was operated over one year ago.

TABLE 3

Response of pancreatic transplant on the application of acid to an intestinal transplant of the jejunum

Dog. "Airedale"

FROM	TO	TIME	PROCEDURE	AMOUNT	REMARKS
		hours		*cc.*	
10:00	12:30	2½	Control, continuous secretion	0.95	
12:30	1:00	½	Applied N/10 HCl to transplant	0.62	Latent period* 6 minutes
1:00	1:30	½	"Recovery"	0.65	
1:30	2:30	1	"Recovery"	0.74	

* Latent period had to be approximated by observing an increase in the number of drops.

The application of hydrochloric acid to the intestinal transplant. It should be pointed out that when the acid was applied for the first time that food had not been in contact with the intestinal transplant for over two months, and that the intestinal transplant was perfused with warm water prior to the application of acid. In these experiments we have used only N/10 and N/20 HCl.

Our results (example in table 3) show that when N/10 or N/20 HCl solution is applied to the intestinal transplant, the pancreatic transplant secretes. The stimulation is not as marked as that which occurs on the application of acid to a Thiry fistula of the jejunum. The latent period is usually longer. This is to be expected because of the decreased blood supply of the intestinal transplant.

DISCUSSION. The use of a Thiry-fistula-pancreatic-transplant preparation to determine whether or not the application of acid, or other substances, to the intestinal mucosa causes the formation of a hormone which excites the pancreas to secrete has the following objections: 1, the

application of the solutions might cause the absorption of secretagogues resulting from the autolysis of cellular detritus in the Thiry fistula; 2, the application of acid and other substances to the intestinal mucosa excites a flow of bile and gastric juice (Ivy and McIlvain, 1923). Nervous reflexes are ruled out because the pancreatic transplant is extrinsically denervated. Since our results show that the Thiry fistula can be washed with water without exciting the pancreatic transplant, we do not believe it at all likely that excitation when it occurs can be accounted for by the absorption of secretagogues. (This, however, does not necessarily mean that secretagogues play no rôle in exciting the pancreas in normal digestion.) Also, since acid applied to the Thiry fistula after ligation of the common bile duct and after atropine injection still causes excitation of the pancreatic transplant, we believe that the application of the acid can only lead to stimulation of the pancreatic transplant by causing the intestinal mucosa to add something to the blood—not the lymph because the latent period is too short.

We believe that our observations on the application of acid to the intestinal transplant with the result that the pancreatic transplant secretes must be considered as crucial evidence that the intestinal mucosa can give off a hormone for external pancreatic secretion.

We believe that this mechanism is physiological and that it occurs in normal digestion. However, we do not care to discuss that question at this time.

The fact that in some experiments 1.5 mgm. of atropine prevented N/20 HCl applied to the Thiry fistula from exciting the pancreatic transplant seems to be a discrepancy, since secretin action is not materially influenced by atropine. But it is to be kept in mind that secretin solutions are not pure and the experiments are performed under anesthesia. The above observation falls in line, however, with what is known concerning the action of atropine on gastric secretion. Atropine (1.5 mgm.) abolishes the gastric secretory response to a meal, but does not abolish the response to gastrin or histamine unless large doses (of atropine) are given (Keeton, Luckhardt and Koch, 1920). It has been observed that atropine changes the response of the pancreas to such excitants as soap and fatty acid in unanesthetized animals (Babkin, 1914). In a previous paper (Farrell and Ivy, 1926) we found that when 150 cc. of N/10 HCl were given by stomach tube to animals with a pancreatic transplant, 1.5 mgm. of atropine did not diminish the response of the transplant to the acid. In this experiment we believe that atropine had no effect because the pancreas was being stimulated maximally; but in the experiments cited in this paper the acid was applied to only a twelve inch loop of jejunum, and hence a submaximal stimulus was operating, which would be more likely to be influenced by the toxic action of atropine.

SUMMARY

1. A method for transplanting a loop of intestine under the skin is described.

2. Application of water to a Thiry fistula of the jejunum does not excite secretion in a pancreatic transplant.

3. The application of acid (N/10 − N/20 HCl) to a Thiry fistula of the jejunum excites secretion in a pancreatic transplant. This occurs after ligation of the common bile duct and after atropine (1.5 mgm.) injection. Atropine, however, decreases the response.

4. The application of acid (N/10 − N/20 HCl) to transplanted loops of jejunum excites secretion in a pancreatic transplant.

5. We believe that this evidence proves that acid causes the intestinal mucosa to give off a hormone for external pancreatic secretion.

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THE EFFECT OF THE PERICARDIUM ON CARDIAC DISTENTION AS DETERMINED BY THE X-RAY

J. A. WILSON AND WALTER J. MEEK

From the Laboratory of Physiology, University of Wisconsin, Madison

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The pericardium has long been known to play a very important rôle in certain abnormal conditions such as acute and chronic effusions, or in cases of wounds of the heart, where considerable amounts of blood have accumulated in the pericardial cavity. Experimental reproduction of these conditions by Cohnheim (1889), Francois Franck (1897), Lewis (1908), and finally Kuno (1917), have led to the common conclusion that as intrapericardiac pressure increases, venous pressure rises to an identical degree, while arterial pressure and output fall correspondingly.

That this surrounding envelope might also restrain the normal heart under certain conditions was first suggested by Barnard (1897–98) who showed that it was absolutely inextensile and that not only would a cat's heart rupture at a lower internal pressure with the pericardium removed, but that also at a given venous pressure level the filling of such a heart was about 100 per cent greater when freed from its pericardium. In living anesthetized animals in whose pericardia slits had been made, the heart would herniate from the sack with each diastole. Barnard suggested that the function of the pericardium was mainly that of supporting the right ventricle and of preventing relative incompetence of the tricuspid valve. Observations somewhat similar were also made by Hill (1898–99).

Perhaps the most comprehensive studies of a restraining action of the pericardium have been made on the heart-lung preparation. Evans and Matsuoka (1914–15) demonstrated that the gaseous metabolism of the heart is quite proportional to the diastolic volume or the length of its fibers. Removing the pericardium brings about a drop of venous pressure, if it is not already very low, and a coincident increase in gaseous metabolism and output from the heart. It is interesting to note that the changes observed by them were always more pronounced, on opening the pericardium, the more nearly the previous output with intact pericardium had approached a maximal value.

Kuno (1915–16) also using the heart-lung preparation, noted that when the pericardium was opened, venous pressure fell and this was accompanied by increased blood pressure and output. Of especial interest to

us is his observation that not only at high but also at low venous pressures there was a fall of venous pressure on opening the pericardium. He found himself at a loss to explain this unexpected result, and suggested that it was probably a consequence of using the open-chest method, in which he thought that the heart dropped back into the chest pulling the pericardium down over the auricles so that venous filling was hindered. Then when the pericardium was removed the unhampered filling of the heart allowed an increase in diastolic heart size. As we shall see, another factor probably lay at the bottom of this unexpected finding. Kuno found, further, that although the output of the heart was about 25 per cent greater as a maximum limit, after removal of the pericardium, this increased work involved damage to the heart in the form of hemorrhage into the muscle and hence the optimum venous pressure was far less for a pericardiectomized heart than for a normal one. His conclusion was, then, that the pericardium plays a protective rôle in cardiac function, guarding against damage which might follow an excessively high venous pressure. He considered the presence of this structure necessary for the unimpaired working of the heart in normal life.

That whatever restraining action the pericardium may exert is not, however, essential for life or even for a normal existence, is shown by the fact that there are many authentic cases on record in man, where there has been partial or complete congenital absence of this structure. Moore (1925) has recently reviewed the literature in this field and states that the absence of clinical symptoms and the normal and healthy appearance of the heart in the great majority of instances would lead to the conclusion that the existence of the pericardium is not necessary for the unimpaired function of the heart in normal life. It doubtless does protect, however, against the formation of adhesions from the heart to neighboring parts.

These same conclusions are borne out by the fairly large series of attempts to determine the effects of surgical removal of the pericardium in animals or man. The earliest of these was made by Amerio (1894) and the most recent by Beck and Moore (1925). Some of the investigators claimed to have observed a slight degree of functional impairment or anatomical change in the heart as a result of such removal, but the majority of them could observe practically no difference between operated and normal animals. Yamada (1916–17) concluded that dilatation of the heart does not follow pericardiectomy because of an increased pulse rate which raises cardiac tonus and lowers venous pressure. Beck and Moore (1925) found no functional impairment on removal of the pericardium in dogs, but as to a subsequent dilatation, studied by the x-ray method, their results were inconclusive.

Felix, a German surgeon, concluded from a series of experiments that valvular lesions which normally are compensated by dilatation of parts

of the heart, such as mitral or aortic insufficiency, are more readily compensated when the restraining action of the pericardium for dilatation has been removed. He recommended this procedure in selected cases in man.

In recent years Starling and his followers (1922, 1927) have advanced the important conception that dilatation is a perfectly normal process by which the cardiac muscle fibers attain the length necessary for developing the extra force of contraction required to meet the demand of exercise or incompetence of valves. Naturally, the pericardium eventually enters in as the limit to which this dilatation can occur, and both Starling and Bainbridge (1923) have laid considerable stress on the compensatory dilatation to these pericardial limits, after which further increase in work must be done through increased rate.

Henderson (1923) and Wiggers (1923) both incline to the view that all volume changes of the heart fall far short of the limits set by the pericardium. Beck and Holman (1925) have, however, shown in a transfused animal that the pericardium does limit the dilatation due to plethora.

In some of the authors' unpublished experiments, directed toward another end, the restraining action of the pericardium has also been noted. In dogs subjected to chloroform anesthesia, the silhouette area of the heart is markedly greater than that of the normal animal regardless of whether the heart rate increases or decreases. In the same animal previously treated with morphine so that the heart rate was slow enough to allow a maximal filling, the heart size during chloroform anesthesia was not further appreciably increased. Evidently in this latter case the pericardial limits had been reached.

From this brief survey of the literature one can deduce that the general opinion of most investigators is that under certain conditions the pericardium does enter in as a restraining influence against too great dilatation, but the problem is not clear as to the exact limits of this restraint. The present paper reports an attempt to establish these limits in terms of venous pressure.

METHODS. The procedure followed in determining changes in the size of the heart has been the x-ray method used in this laboratory on several other problems (Meek and Eyster, 1921, 1922). The distance from the Coolidge tube to the plate, which was placed in a carrier immediately beneath the animal, was kept constant at one meter. This rendered all exposures in any one experiment comparable. Exposures were always made by one or two flashes long enough to insure diastolic size, and placed toward the end of inspiration in order that the apex might be above the diaphragm. Although Skavlem (1922) has developed a formula for converting the x-ray silhouette area of the heart into terms of volume, in our experiments sufficient information is afforded from a direct comparison of the changes in area of the heart shadow, since the same relationships

of tube, animal and plate were preserved throughout. Measurements of this shadow were carefully made with a planimeter. For cardiac x-ray measurements the dog is much more satisfactory than man, since the outline of its heart is relatively much freer from diaphragm and liver shadows. The only chance for real error in measurement is at the base, and if the same contour is preserved for a given width, this may be almost entirely eliminated. Successive pictures on the same animal may be taken well within a variation of 5 per cent. In this we agree with the recent conclusion of Stewart (1927).

In our experiments 41 dogs in all have been used, all procedures being carried out under morphine-ether anesthesia. Both external jugular veins were exposed. Into one of them was inserted a long metal sound which reached to the level of the auricle and was connected with a water manometer and reservoir filled with 2 per cent sodium citrate in physiological salt solution. Into the other jugular was placed a large-bored glass cannula attached to a long rubber tube leading to an elevated reservoir of 0.9 per cent NaCl. This reservoir was kept immersed in a water bath of sufficient temperature to deliver the fluid from the cannula at about 37 or 38°C. Blood pressure and heart rate were recorded with a mercury manometer attached to the femoral artery.

To study the restraining effect of the pericardium on the heart, recourse was had to two somewhat different procedures.

1. Without any thoracic operation, the first x-ray picture was taken on the anesthetized animal, at the existing initial venous pressure. Then by saline injections through the left external jugular vein the venous pressure was raised and x-ray pictures were taken at selected venous pressure levels from low up to fairly high values. The venous pressure was always maintained for at least 15 to 20 seconds at any level before taking the picture so that conditions might become stabilized. Then venous pressure was allowed to fall gradually back to its original low level. Meek and Eyster (1922) have previously shown that an artificially induced plethora has only a transient effect on the diastolic heart size, due to opening up of unused capillary regions, as well as to some amount of transudation where the injection has been large.

Next, under artificial respiration, the chest was opened and the pericardium thoroughly incised to allow complete freedom of the heart. The chest was then reclosed and normal spontaneous breathing once more established. Several experiments included a registration of intrathoracic pressure before and after this procedure, which showed no appreciable change in its value.

A second series of x-ray pictures was then taken, at the same venous pressure levels used before the operation. In this way accurate deductions were made possible as to the effect of the pericardium at each level of venous pressure.

2. The second procedure differed from the first only in that the chest was opened before taking any pictures, and two cutting ligatures were laid in the pericardium, extending up through a small hole made at the apex to two more small holes made, one on each upper lateral border of the pericardium. The two ends of each ligature were then threaded into a needle and carried out through the lower anterior margin of the thoracic wall to the outside. The exit holes for the ligatures were about three or four inches apart. The chest was now reclosed and spontaneous respiration reëstablished.

The first set of normal records at various venous pressures was now taken, and then the pericardium was opened in the frontal plane by applying a firm sawing motion to the ligatures on each side. When the pericardium had been cut through, the second series of x-rays was obtained at the same venous pressures used before.

In the last seven experiments where the second procedure was followed, while the chest was open for the purpose of laying the ligatures, the fibrous attachments of the pericardium to the central tendon of the diaphragm were severed.

At the end of the experiment, the dog was killed, and the chest opened in order to determine the efficacy of our ligatures in opening the pericardium. In all but one or two experiments a complete removal of this structure had been effected.

No less than six pictures were taken of every heart, while the number usually ran from 10 to 15. Hence this study includes a total series of between 400 and 500 heart pictures at varying venous pressure levels.

Several other series of experiments were run that had a definite bearing on our problem. In five animals venous pressures were determined with the chest intact, and x-ray pictures were taken at each pressure level. The animals were then killed under the anesthesia and the pericardial cavity connected to a water manometer and reservoir. X-ray pictures were next taken of the pericardial area at ascending intrapericardial pressures in order to determine the maximal limit of heart size which the pericardium could allow. In two experiments the pericardium was subsequently removed and the heart itself distended by raising intrapericardiac pressure to find out whether the heart could dilate beyond the limits set by the pericardium.

In some cases, two sets of preliminary experiments were run on dogs before the day of the final operation. The first such experiment was to measure the area of the heart shadow in a normal animal just before and during moderate ether anesthesia. The results of these experiments showed us that in the majority of animals ether itself does not change the area of the diastolic heart shadow. The second preliminary trial was a set of similar x-ray pictures before and after chloroform anesthesia, simply

for the purpose of comparing the large heart size always produced by chloroform with the size found at various venous pressure levels during the subsequent major experiment.

RESULTS. Our results have been arranged as far as possible so as to answer three main questions:

1. Is an uncomplicated restraining action of the pericardium always to be found?

2. At what venous pressure levels is such restraint first evidenced?

3. What venous pressure is required to distend the heart to the limits set by the pericardium?

By way of introduction it may be mentioned that a large series of experiments in this laboratory has demonstrated that ordinarily moderate ether anesthesia has in itself no appreciable effect on the diastolic size of

TABLE 1

The effect of ether on heart size in decerebrate dogs

DOG NUMBER	WITHOUT ETHER		WITH ETHER		PER CENT CHANGE IN AREA
	V.P. in cm. H_2O	Heart area in square centimeter	V.P. in cm. H_2O	Heart area in square centimeter	
3	+2	67.5	+2	67.8	+0.5
	+10	72.9	+10	71.9	−1.4
7	−3	46.8	−3	46.5	−0.7
	+5	58.1	+5	58.5	+0.7
10	−3	36.9	−3	36.2	−1.9
	+5	43.0	+5	44.6	+3.7

the heart, as measured by the x-ray, when secondary changes due to altered venous pressure are taken into account. In table 1 may be seen figures showing that at any given venous pressure the heart size is unchanged by moderate ether anesthesia and the ability to dilate farther with a rising venous pressure is not altered. These results are mentioned to justify our attacking the problem on animals under ether anesthesia. It is of course recognized that excessive concentration of ether may definitely damage the heart and bring about dilatation just as always happens under chloroform, but such heavy concentrations were carefully avoided in our experiments.

1. *Is an uncomplicated restraining action of the pericardium always to be found?* Very early in this present series of experiments it was noticed that not only at high venous pressures where it might be expected, did the pericardium restrain the heart, but that in many experiments the same

result was found at low initial venous pressures. Apparently, no matter what procedure was followed 40 to 50 per cent of the experiments showed the heart size appreciably larger even at low venous pressure levels, after the pericardium had been removed. The first half of table 2 shows typical examples of the marked variability of these data.

Practically the same condition in the heart-lung preparation was found by Kuno (1915) who noted a drop of venous pressure upon pericardiotomy, even with small venous inflow, and this he interpreted as a necessary consequence of the open-chest experiment. However, our results now showed the same thing in many cases with the chest closed. It was felt to be

TABLE 2

Heart size before and after pericardiotomy at low venous pressures

NUMBER OF EXPERIMENT	V.P.	AREA BEFORE PERICARDIOTOMY	AREA AFTER PERICARDIOTOMY	PER CENT CHANGE
Pericardial attachments intact				
14	−2	40.4	45.0	+11.4
22	−2	41.8	41.6	−0.5
25	−4	50.4	51.4	+2.0
27	−2	52.2	59.0	+13.0
29	−2	41.4	40.2	−2.9
30	−1	49.3	56.9	+15.4
34	−3	52.4	54.5	+4.2
Pericardial attachments severed				
35	−2	41.4	42.0	+1.5
36	−2	58.0	57.6	−0.7
37	−2	49.1	49.8	+1.4
38	−3	50.0	48.5	−3.0
39	−2	77.0	76.6	−0.5
40	−3.5	43.6	43.4	−0.5
41	−1	50.0	49.8	−0.4

inconceivable that this could be a real effect of the pericardium in view of its inelasticity. Hence a search was made for some sort of a complicating factor.

Stephens (1922) has reported that intrapericardial pressure has a value of about minus 4 cm. of water in the usual corpse at autopsy, with the chest open, which suggests a strong pull on the pericardium by its diaphragmatic attachments at the same time that the heart contracts down in rigor mortis. This fact together with the observation made in our own experiments that the pericardiodiaphragmatic attachment is quite variable in dogs, being very firm in some and quite easily separated in others, led

us to suspect that the variability of our results at low venous pressures might easily be due to tension from these attachments.

Hence, in the last seven experiments of our series when the chest was open, not only were the ligatures laid in the pericardium but the latter was thoroughly separated from the diaphragm, and the chest then reclosed. The results of these experiments are shown in the second half of table 2, and are surprisingly uniform. It is then evident that the variability in data and the restraint of the pericardium at low venous pressures are due to the diaphragmatic attachments.

That our results are not due to changes in rate may, we think, be ruled out for the rate changes are not related to changes in area. Moreover, one of us (Meek, 1924) has shown that if venous pressure is artificially maintained at any given level, the heart rate may be advanced to values around 200 per minute without influencing diastolic heart size. Since in our experiments, all comparisons of heart size have been made at the same venous pressure, rate changes have not been significant.

The mechanism by which we assume the diaphragm to influence pericardial restraint on the heart at low venous pressures is by drawing the upper lateral walls of this sack down over the auricles and inhibiting venous filling at these low pressures. Hence when the pericardium is opened, venous filling is unduly augmented and we have recorded larger heart sizes. That some dogs show this effect and others do not, must doubtless be due to the variable anatomical nature of this attachment.

2. *In hearts uncomplicated by attachments to the diaphragm, at what venous pressure level does the pericardium begin to restrain diastolic heart size?* The phrase, uncomplicated by attachments, must now be added to this second question, for the previous discussion has shown that even at the lowest venous pressures the pericardium in the intact chest may exert some restraint on the heart. In table 3 are given the changes in heart size at each venous pressure level in 7 experiments where the pericardial attachments have been severed. The per cent changes have been averaged and are presented in figure 1. These data show that when venous pressure reaches 0 cm. of water, there is a very slight beginning restraint exerted by the pericardium, which attains a distinctly elevated level at a venous pressure of +2 cm. of water, and from then on increases almost proportionally to the rise in venous pressure. One might on first thought think it strange that this pericardial limitation should not appear maximally when it appears at all, since the tissue of this sack has been shown to be perfectly inelastic. However, if one remembers that the general shape of the pericardium is that of a roughly truncated pyramid, it is not hard to see that as the heart dilates and becomes more globular, some adaptation of this irregular shaped sack must occur before it completely checks dilatation. It is probably this accommodation which

TABLE 3

Experiments uncomplicated by pull of pericardio-diaphragmatic attachments

Heart areas at various venous pressures before and after pericardiotomy

EXPERIMENT NUMBER	V.P. IN CM. H_2O	AREA BEFORE REMOVAL	AREA AFTER REMOVAL	PER CENT CHANGE IN AREA
		sq. cm.	*sq. cm.*	
35	−2	41.4	42.0	+1.5
	0	44.9	46.7	+4.0
	+2	46.9	51.6	+10.0
	+5	49.0	53.0	+8.2
	+10	51.8	59.6	+15.1
	−2	40.3		
36	−2	58.0	57.6	−0.7
	0	65.0	66.3	+2.0
	+2	68.1	70.4	+3.4
	+5	70.0	72.5	+3.6
	+10	69.8	77.4	+10.9
	+15	71.3	80.5	+12.9
	−2	57.8	62.2	
37	−1.5	49.1	49.8	+1.4
	0		53.0	
	+2	51.3	55.4	+8.0
	+5	53.7	60.4	+12.5
	+10	56.1	60.6	+8.0
	+15	55.7	61.8	+11.0
	−1.5	47.2	52.2	
38	−3	50.0	48.5	−3.0
	0	53.8	54.9	+2.0
	+2	54.1	66.5	+22.9
	+5	54.4	69.1	+27.0
	+10	54.4	72.0	+32.4
	+15	55.0	71.5	+30.0
	−3	50.9	49.7	
39	−2	77.0	76.6	−0.5
	0	80.3	80.6	+0.4
	+2	84.0	84.6	+0.7
	+5	85.2	87.2	+2.3
	+10	87.1	90.4	+3.8
	−2	77.0		
40	−3.5	43.6	43.4	−0.5
	−1	46.7	46.4	−0.6
	+2	47.0	51.3	+9.1
	+5	48.5	57.1	+17.7
	+10	50.0	58.0	+16.0
	−3.5	44.9	48.1	
41	−1	50.0	49.8	−0.4
	+2	56.3	56.0	−0.5

explains, in table 3, the fact that with the pericardium intact, the restraint seems almost complete at moderate venous pressures, but a higher venous pressure may still give a slightly large heart size. This process of course ceases as soon as the heart entirely fills the pericardium.

3. *What venous pressure is required to stretch the heart to the limits set by the pericardium?* This has been determined in two ways. First, by inspection of table 3 it is seen that as venous pressure rises, the heart size

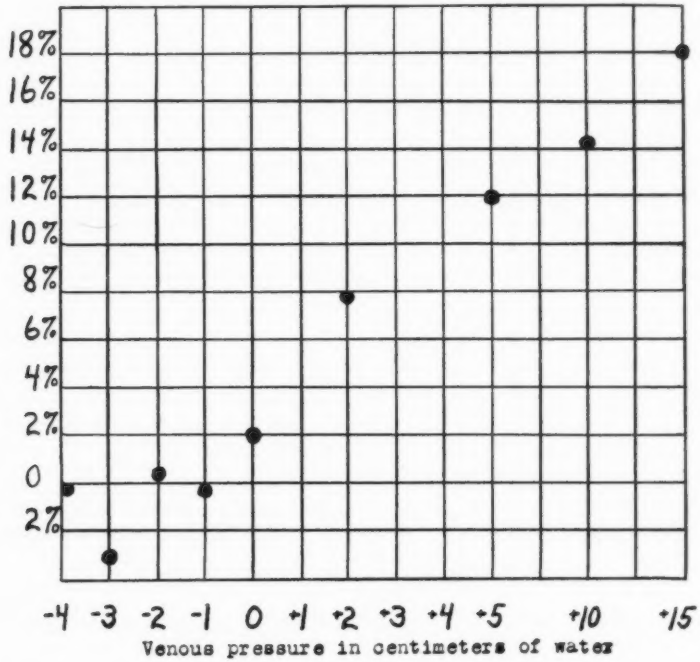

Fig. 1. Graph showing per cent increase in area over control after pericardiotomy. Average values for 7 experiments in which pericardial attachments were severed.

reaches a point beyond which further increase in pressure does not cause further distention of the heart. An average of all our experiments in which the pericardial attachments were intact shows this optimum of venous pressure to be at +7 cm. of water. In the series of experiments carried out with pericardial attachments severed, it is about 8.5 cm. of water. This slight difference is probably more apparent than real, due to the smaller number of experiments included in the latter series.

The second determination of this level consists of experiments of the type contained in table 4. The venous pressure level at which further increase in heart size ceased with the pericardium intact was determined, and then the animal was quickly killed and a cannula inserted into the pericardium, which was subjected to varying distending pressures, at

TABLE 4

Showing venous pressure necessary to fill the pericardium

V.P. IN CM. H_2O	HEART AREA	PERICARDIAL AREA-SQUARE CENTIMETER AT VARIOUS INTRA-PERICARDIAL PRESSURES	
	sq.cm.		
−2	59.4		
0	61.5		
+5	68.5		
+8	71.0		
+50		70.5	
−1	54.5		
+2	57.8	58.4	
+5	59.0	59.7	
+10	58.2	59.7	
+14	60.8	59.9	
+40		60.3	
−5	28.1		
0	33.0		
+5	32.9	31.1	Cannula in heart after pericardiotomy. Heart area in sq. cm. 40.8
+10	33.5	30.9	
+15	33.3	32.6	
+5		40.5	Cannula in heart after pericardiotomy. Heart area in sq. cm. 50.9
+15		43.0	
+40		43.0	
−4	38.5		
0	46.7		
+5	47.4	46.4	
+15		46.8	

which x-ray pictures were taken. Table 4 shows that at venous pressures of 8 and 5 cm. of water in the first two experiments, and at 0 cm. in the next two, the pericardium was completely filled, for the pericardium itself when subjected to pressure could not be further distended. That these experiments should give a low level for optimal venous pressure is to be expected, since where the heart has stopped beating all "tonus" due to

contraction remainder has disappeared and less distending pressure is needed to make the heart fill the pericardium. That this check on further distention is really due to the pericardium is seen in the two experiments of table 4 in which after removal of the pericardium the heart could be distended far beyond its previous limit. We conclude then, that in the living dog, a venous pressure of from 5 to 8 cm. of water is sufficient to distend the heart to the limits set by the pericardium.

Just how far the conclusions arrived at can be carried over to the human it is difficult to say. It seems very probable that, since the pericardio-diaphragmatic attachments in man are very broad and usually very firm, in man also there may be the diaphragmatic-pericardial restraint on venous filling even at low venous pressures. Bardeen (1918) states that at the height of a deep inspiration the heart, or at least the heart shadow, may be smaller than normal owing to the pull of the diaphragm. He observed this in a few cases where the subject lay in the prone position, but failed to find it with the subject in the sitting position. Felix (1925) has reported an observation by Sauerbruch to the effect that on operative opening of the pericardium in man, the heart visibly dilates. Beyond that rather unsatisfactory statement, no further observations, especially of x-ray studies after human pericardiectomy, are known to the writers. According to several surgeons, operative removal of the pericardium in man is advantageous in cases where compensatory dilatation is desired.

These data also support the earlier observations of Meek and Eyster (1922) as to the "critical" venous pressure level, i.e., the level beyond which further increase in heart size and output ceases. They found this value, in the intact animal, around 150 mm. of water of "effective" venous pressure, assuming intrathoracic pressure to be minus 50 mm. of water. In those of our experiments in which intrathoracic pressure was measured, this was found to average nearer minus 80 mm. of water. Adding this value to the venous pressure needed to distend the heart to the pericardial limits, we find an "effective" venous pressure of 160 mm. of water. This is considerably lower than the figures found as "critical" by Wiggers and Katz (1922), 250 to 310 mm. of water in open-chest dogs on whom the cardiometer was used, but is much higher than the same level set by Henderson and Barringer (1913) at 50 mm. of water.

Whether these data throw any light on the question as to physiological dilatation and the restraint exerted by the pericardium as emphasized by Starling and Bainbridge, is questionable. Certainly we can say that in the dog, as venous pressure rises the diastolic heart size does increase up to a maximum which is reached at a level of venous pressure quite probably attained in severe exercise. Once this limit has been reached, naturally any further increase in oxygen supply to the active muscles must be

effected through an increased heart rate, and an increased coefficient of utilization.

CONCLUSIONS

1. In the dog, an apparent restraining action of the pericardium at low venous pressures (from −4 to 0 cm. of water) is due to the tonus of the diaphragm affecting the heart through the pericardio-diaphragmatic attachments.

2. With these attachments severed, the uncomplicated restraint exerted by the pericardium on diastolic heart size is first noticed at a venous pressure of 0 cm. of water, and has reached an appreciable magnitude at 2 cm. of water. This restraint increases gradually until the heart fills the pericardium, and then becomes more and more apparent the further venous pressure is elevated.

3. At a venous pressure of about 8 cm. of water, or at an effective venous pressure of 15 or 16 cm. of water, the dog's heart completely fills the pericardium.

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A STUDY OF THE BULBO-SPINAL REFLEXES IN DOGS AND CATS UNDER BARBITAL ANESTHESIA

J. FRANK PEARCY AND M. M. WEAVER

From the Departments of Physiology of The University of Chicago and West Virginia University

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Barbital has been extensively used as a hypnotic. Tatum and Parsons[1] in 1922 called attention to its value as a general anesthetic. We used barbital-sodium, since it is more soluble than barbital and does not require neutralization. One gram dissolves in approximately 6 cc. of distilled water. It requires considerably more physiological saline to dissolve it, presumably because of the effect of the common sodium ion.

The dosage of barbital sodium varies with the method of administration, the age and the size of the animal. The following quantities will usually induce good surgical anesthesia in adult dogs of average size: per os, 0.3 gram per kilo body weight; intramuscularly or intraperitoneally, 0.27 gram per kilo; intravenously, 0.20 to 0.25 gram per kilo. The dose has to be slightly increased for very small, and decreased for very large adults. Very young and very old dogs require slightly smaller quantities. Cats require slightly smaller quantities than dogs (0.25 gram per os).

Per os, barbital sodium induces surgical anesthesia in 30 to 60 minutes: intramuscularly or intraperitoneally, in 20 to 40 minutes; intravenously, in 10 to 20 minutes. The intramuscular method is preferable.

If the barbitalized animal shows spontaneous movements it may be quieted by injecting more barbital or by the intravenous or subcutaneous injection of $\frac{1}{8}$ to $\frac{1}{4}$ grain of morphine sulphate. After an animal has been under barbital anesthesia for several hours it may become restless due to the reflexes set up by a distended bladder or colon. In such cases the colon or bladder should be emptied. A restless preparation is almost useless for the study of the reflexes. Somatic reflexes are difficult to obtain. Even the knee jerk may be barely elicited. Spontaneous gastro-intestinal activity as well as reflexes to the gut may be completely abolished. If the animal is too "deep" under the anesthesia it can frequently be made suitable for experimental work by giving from $\frac{1}{200}$ grain to $\frac{1}{100}$ grain of eserine and from $\frac{1}{2}$ to 1 grain of caffeine. When the animal is under surgical anesthesia it can be used at once, but better results are obtained if it

[1] Tatum and Parsons: Journ. Lab. Clin. Med., 1922–23, viii, 64.

is permitted to "sleep" (in a warm place) for from two to four hours before experimentation.[2]

RESULTS. *Skeletal reflexes.* The knee jerk is very brisk. It is practically as brisk and as easily obtainable as in chronic transected preparations. A light tap on the patellar ligament with one finger is sufficient stimulus to produce a vigorous reflex. The response can be obtained hour after hour practically without change.

The flexion reflex of the hind legs is easily elicited. A gentle pinch of the foot frequently produces a sustained and maximal withdrawal of the foot. This reflex can be obtained repeatedly.

Gentle pressure applied to the bottom of the foot causes a marked extension reflex of the foot in many preparations. The reflex is easily elicited and lasts practically as long as the stimulus is applied. In some cats it was found that squeezing a hind foot caused a very brisk double extension reflex. When the flexion reflex is obtained by noxious stimulation of one foot extension of the other leg occurs. This crossed-extension reflex is easily elicited. The abdominal reflexes have been obtained in some preparations. When present they are easily elicitable and are brisk.

A good scratch reflex was never obtained. It was frequently possible, however, tc produce a series of rhythmic flexion-extension movements of the hind leg, but the foot was never raised to the point of stimulation.

Viscero-somatic reflexes. Marked and sustained increased tonicity of the abdominal muscles was obtained by gently stroking the parietal peritoneum with a finger or by gentle distention of the small intestine. As the innervation of the parietal peritoneum is probably somatic, only the latter reflexes produced from the viscera should be classed as viscero-somatic.

By gentle distention of the gut one can usually produce an increased tonus of the anti-gravity muscles. The four legs extend rigidly and in the cat the head and the tail are raised. In some preparations, instead of "extensor rigidity," running movements of the hind legs are obtained.

Visceral reflexes. Reflexes from one portion of the gut to another were obtained. The distention balloon was placed in any portion, as for example, in the ileum, and the recording balloon was placed in any other portion, such as the duodenum. The intestine was severed between these portions in order to be certain that we were dealing with a true spinal cord reflex. A mild stimulus was sufficient to produce immediate and very marked fall in tonus and complete cessation of motility of the gut. When a distention balloon of from 2 inches to 2.5 inches in length

[2] Other barbituric acid derivatives were tried (luminal, amytal, ipral). For our work, these had no advantage over barbital-sodium and they are more troublesome to use.

was used, a distention of from 16 mm. to 20 mm. Hg was sufficient to produce the reflex.

The stimulus also caused blood pressure changes. Usually the systolic pressure would rise 30 mm. to 60 mm. Hg and remain there for several minutes. Marked splanchnic vaso-constriction also resulted, as evidenced by controlled oncometer records of the small intestine, the kidney and the spleen. Peripherally the response was a vaso-dilatation. Occasionally the peripheral dilatation exceeded the splanchnic constriction and a fall in systolic blood pressure resulted.

Distention of the intestine with a pressure of 16 mm. to 20 mm. Hg also affected the respiration center. Usually a few deep inspirations resulted. Frequently, however, the rate was markedly accelerated. In either case there was increased aeration as evidenced by the relative apnea that followed release of the stimulation.

Vomiting occasionally resulted from intestinal distention. It did not occur regularly and vigorous stimulation was required to elicit it.

We were surprised to find that after complete transection of the spinal cord under barbital "spinal shock" was frequently not observed for a period of from a few minutes to half an hour or more. For instance, the spinal cord was cut at the 12th thoracic, the 6th thoracic and the 7th cervical region, and the knee jerk, which was originally very brisk, was not demonstrably diminished during a period of over an hour after the transections. The flexion reflex was not diminished until about thirty minutes. In one cat which showed a vigorous double-kick response to stimulation of the hind foot, the cord was transected in the midthoracic region. The reflex was obtained without any depression immediately (15 seconds) after the transection. It was easily fatigued, but when given sufficient rest periods, it was found that the reflex gradually diminished and was practically lost in thirty minutes. The extensor-thrust reflex and the crossed-extension reflex were usually abolished almost at once.

The viscero-somatic reflexes were only slowly lost. Of the visceral reflexes, the inhibition of the gut was not affected if in the transection essential local cord pathways were not cut. The Lovén reflex is not interfered with if the blood pressure is maintained.

In none of these cases did the reflexes disappear immediately and then return. On the contrary, after transection the reflexes were present immediately and disappeared gradually.

Barbitalizing decerebrate animals does not decrease the reflexes. It thus seems that barbital in anesthetic doses affects mainly the cerebrum. The blood pressure and body temperature remain normal for 24 to 36 hours.

SUMMARY

Barbital, in quantities sufficient for surgical anesthesia, causes little or no depression of the bulbo-spinal reflexes. The properly barbitalized animal is equal and in some respects superior to the decerebrated animal for the study of the bulbo-spinal reflexes. Spinal cord transection under barbital delays the onset of spinal shock, as judged by the reflex response of the spinal animal.

We wish to express our thanks to Prof. A. J. Carlson for generous help and counsel, and to Prof. A. B. Luckhardt for valuable suggestions.

NOTES ON THE REFLEXES OF PUPPIES IN THE FIRST SIX WEEKS AFTER BIRTH

ALICE M. BAHRS

From the Rudolph Spreckels Physiological Laboratory of the University of California

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In a study of the behavior of young puppies in the first six weeks after birth I have found certain reflexes which seem not to have been described or which differ from the reactions of older dogs.

1. *The pinna reflex.* The pinnae of pups during the first two weeks of life differ considerably from those of adult dogs in that they are thicker and much more rigid. The thickness and rigidity are at this age characteristic even in varieties of the "flap-eared" type. As a matter of fact most of my observations were made upon mongrels which turned out to be of the flap-eared variety. For this reason they are all the more striking.

Movements of the pinnae occur much more frequently in very young pups than in adults. Especially during sleep, jerking movements of the ears occur frequently, as well as of other parts of the body.

Sherrington studied pinna reflexes in intact and in decerebrate cats and also in other animals, but these were responses to direct stimulation of some part of the pinna itself. The reactions herein described occurred when the position of the head in space was changed, and were not the result of direct stimulation of the pinna. The locus of the excitation has not been ascertained; it may be in the labyrinth.

When a pup is placed upon its ventral surface in the hands and is then rotated through an angle of 90° around its horizontally placed longitudinal body axis the pinnae assume a characteristic position. The side of the head is now parallel to the floor. As soon as the lateral tilting of the body begins, the pinna on the upper side of the head rises, dorsally, until its dorsal surface is pressed against the side of the head. This position completely exposes the external auditory meatus, which normally is covered by the pinna. In its new position, the pinna is rigid to the touch. The pinna, on the lower side, takes a position perpendicular to the side of the head and is also rigid. Whether the pups are asleep or awake, the reaction takes place, and violent struggling does not hinder it. During the time the reaction is in progress, it is frequently necessary to hold the muzzle in order to keep the head parallel with the floor. The reaction of the pinnae also takes place when the head only is placed in the lateral position, even if the trunk has not been inclined.

A great variation in the time required for the reaction was observed. The time was measured by a stop watch, which was started when the lateral tilting of the body began and was stopped when the upper pinna had flattened itself against the side of the head. The pinna usually began to raise itself in the dorsal direction as soon as the tilting of the body occurred, but the reaction was sometimes not complete until the lateral position of the head had been maintained several seconds or even minutes. Four seconds was the shortest time recorded, while in other cases the reaction took place only after the pup had been held in the above-mentioned position 192 seconds. The usual time was less than one minute.

The reaction could be elicited in the very young pups until the age of one to two weeks after which time the upper pinna no longer flattened itself in the reverse direction against the side of the head, but remained flapped over the external auditory meatus. At this age the pinnae are no longer rigid but have come to resemble in proportions those of the adult.

The behavior of the upper pinna only has been considered in this study; the lower one seems to hang passively in response to the pull of gravity upon it. It was only the upper pinna which executed any movement.

Gravity is not responsible for the reaction of the upper pinna, for part of the movement is in a direction which would be opposed by the weight of the ear. Furthermore the reaction should not cease when the pinnae became larger and less rigid, if this force were the cause. Work has to be done by the ear muscles to raise the upper pinna up to the point where it is perpendicular to the side of the head. After this position has been reached, gravity might make the pinna drop at once against the side of the head, but instead it comes down slowly. The muscular control over the movement of the upper pinna was always observed, whatever the duration of the process.

The reaction can be obtained on the same animal apparently as often as the change of position is repeated, and repetition seems to have no effect upon the time required for the reflex to take place.

2. *The seal reflex.* When two-months-old pups are lifted by grasping them under the forelegs and are held with the body axis approximately vertical a rigidity and a definite posture are observed. The following is, in general, the position taken: The back becomes markedly opisthotonic, the tail points rigidly in the caudal direction, the hind legs are extended completely caudally and the toes spread. In some pups the forelegs are flexed, in others they are laterally extended and rigid. The neck in most cases is flexed ventrally, making an angle of 90° with the body, and the head is extended from the neck so that the neck, throat and lower jaw are in a straight line. Some pups extend the head and neck maximally, bringing the spinal column, neck and head into a straight line except for the opisthotonus of the back. The extended head and neck are sometimes

bent backward in the dorsal direction a few degrees, making an obtuse angle with the spinal column. The pup hangs in this position absolutely motionless and the complete rigidity of the body is maintained as long as it is suspended by the hands under the shoulders. Squealing and struggling are completely inhibited. While suspended some of the pups go to sleep, and then the rigidity relaxes; this, however, does not occur frequently. If the hind feet touch a solid object, as a table, the rigidity of the body and the characteristic position of the head disappear in a few seconds. I have called the above-described reaction the *seal reflex*, because in it the dog has the appearance of a seal.

If pups which are only a few days old are suspended in the manner which in the older animals calls out the characteristic seal reflex a different behavior is seen. The body does not become rigid, the hind legs are not extended, the back is emprosthotonic, the head is not extended although it may be flexed ventrally, and the tail is flexed on the body. The pup squeals and struggles when suspended instead of remaining absolutely motionless, as in the case of older pups. The struggling and lack of rigidity of the animals when suspended continues to be the rule until they are about eighteen days old, at which time the opisthotonic position of the back and the rigidity of the body begin to be observed in some pups; in others the seal reflex does not appear till the age of three weeks or more. An intermediate stage was observed in which the pups hang quietly but show no rigidity.

The reactions of one litter of pups were studied through a longer period. Their behavior began to change at about the age of 23 days, at which time they were found to hang quietly but with very little rigidity. At forty-two days of age complete rigidity and the opisthotonic position of the back were observed. The neck was flexed ventrally with the head extended from the neck but the dorsal position of the neck was not taken. This rigid position was maintained as long as the pup was suspended by the hands under the shoulder blades. It was also seen that during the time a forty-two day old pup was suspended the eyes were fixed, and did not move laterally even if the pup were called. The normal lid reflex took place but there was no movement of the eyeballs, with the result that the eyes had a glazed appearance. When the hind feet were touched to a solid object, the eyeballs were released from the fixed position and the pup looked toward the speaker when called.

I tried placing my hands in different positions under the shoulder blades to ascertain whether the place in which the animal was held had any bearing upon the reaction. A pup which was fifty-eight days old was suspended by grasping the upper part of the forelegs but this did not produce the fixation of the eyes although the body was rigid. When the pup was held by placing the hands around the upper part of the thorax just

posterior to the forelegs, the eyes moved freely in all directions, and the body was not so completely rigid as in the fully developed reaction. I then tried suspending the pup by placing my hands just under the foreleg, where the foreleg joins the body, and the immediate rigidity of the animal and the fixation of the eyes appeared. The latter was the method used throughout for the investigation of this reaction.

Three adult dogs were suspended by placing the hands under the forelegs and in these also a rigidity of the body appeared, but in no case was the rigidity so great as that present in the pups. The back was straight, not opisthotonic as in the pups, and the hind legs were not extended caudally. In one dog a slightly dorsal position of the head and neck was seen, but even in this one the body was not so completely rigid as it would have been in the pups under the same conditions. Fixation of the eyes was observed in one of the adult dogs.

3. *The reaction to rotation.* In young infants Bartels found a compensatory movement of the head and a nystagmus of the eyes during rotation in a horizontal plane around the vertical body axis. An after-nystagmus of the eyes also occurred. In young infants a compensatory position of the eyes but not of the head occurred on rotation during sleep, without an after-nystagmus. Premature infants exhibited the same eye reactions as normal sleeping infants during rotation, that is, a compensatory position but no after-nystagmus was observed. The premature infant developed the regular after-nystagmus slowly if it lived long enough but when the reaction first began to develop it was manifested by a few jerks only. No rotation nystagmus was present in the premature infant. Alexander observed newly born infants for the head and eye reactions during and after rotation. The movement of the head in the direction of the rotation reached its maximum upon the third or fourth rotation, at which time it was from 30° to 40° from the median line. When the rotation ceased the head, in a few cases, was not moved at all but in most cases it was moved to the opposite side. An after-nystagmus of the eyes which continued for fifteen or twenty seconds then took place. During the rotation Alexander found no rotation nystagmus but observed that a compensatory position of the eyeballs was maintained. Infants were usually quiet during the rotation and some slept. If one were crying when the rotation began it usually ceased after a few turns. Magnus found in monkeys a reaction of the extremities to rotation. The animal was held in an upright position with the face toward the experimenter; the back of the head secured so that no head movement could take place. By rotation on a horizontal plane the body of the monkey was so turned that all four extremities and the symphysis of the pelvis were in the direction of the rotation. Novotny observed the compensatory movement of the head in the white rat at the age of seven days.

I first tested the rotation reactions of young pups by placing them on their ventral surfaces upon a flat turn-table. No definite results were obtained. The pups squealed and crawled over the turn-table, apparently not oriented in any way by the rotation. On account of the weakness of pups at this age it was surmised that they did not have strength enough in their neck muscles to move their heads against the friction of the table. For this reason a harness was made by cutting four large holes for the legs in a piece of heavy canvas and putting tapes on the top by means of which the harness could be suspended. The pup was thus hung as in a sling, with the abdomen supported by the portion of the canvas between the fore and hind legs while the head, neck, legs and tail were now free to move.

When two-day-old pups were rotated in this way it was found that a compensatory position of the head occurred in the beginning of the rotation, but after a few turns the compensatory position was given up and the head was bent to the opposite side. Thus if the pup was rotated to the right the head was bent to the left, but later the head swung around past the midline and a strong bend to the right occurred which lasted through the period of rotation and for some moments after stopping. This occurrence of the "after-effect" during the actual rotation could be seen until the pups were from eighteen to twenty-two days old. The age varied in different litters and among individuals of the same litter. After this time the reaction became identical with that of the adult; that is, the after-effect appeared only on cessation of rotation.

Characteristic changes in eye nystagmus and after-nystagmus in relation to age were observed, as well as in the limb positions and in the righting reactions. Report on these is postponed for more exact analysis. It is believed that a further study of the whole complex of reactions will throw light on the development of the proprioceptive mechanisms.

I wish to express my thanks to Dr. Rosalind Wulzen, under whom this study has been undertaken, for helpful guidance and suggestions.

SUMMARY

1. A posture reflex of the pinna in young pups has been described. This reaction disappears within one or two weeks after birth.

2. When pups of a certain age are suspended vertically by support just back of the shoulders a characteristic posture is assumed, which has been named the "seal reflex."

3. The reaction to rotation in young pups differs from that of older animals in that a decided "after-effect" appears during the continuance of the rotation.

STUDIES ON THE VISCERAL NERVOUS SYSTEM

Reflexes from the Gastro-Intestinal Tract to the Eye

J. FRANK PEARCY and THOMAS DYER ALLEN

From the Department of Physiology of the University of Chicago and the Department of Ophthalmology of Rush Medical College

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It is common experience that gastro-intestinal "upsets" may be associated with visual disturbances. Whether the gastro-intestinal upset is the cause of the visual disturbance or vice versa has not been agreed upon. Some have accepted the obvious cause-effect relation, that following unusually large meals or dietary indiscretions visual disturbances not previously apparent become so. Others, on the contrary, assume that the visual disturbance is due to an ametropia. Being unable to account for an ametropia on a reflex or toxic basis they have assigned to it the causative rôle. They claim that the asthenopia resulting from the ametropia produces the gastro-intestinal upset reflexly. To an unbiased observer both explanations are possible, for both conditions may exist independently. It was our purpose to determine whether disturbances *could* be produced reflexly from the gastro-intestinal tract and, if so, by what optical change the visual disturbance was produced.

In our first experiments we used cats anesthetized with ether, and dogs either anesthetized with barbital-sodium or rendered quiescent and analgesic with morphine. The colon was distended with a divulsion type balloon and the eye was refracted with the retinoscope to ascertain if any ametropia could be produced. In a few cases the accommodation changed but the change was never constant or predictable. It could never be certainly attributed to the distention. It was concluded that in dogs and cats ametropia did not result from colon distention.

Assuming either that the reflex might be so delicate that the drugs abolished it, or that it might exist in man but not in lower animals, one of us was used as a subject. Either the colon was distended with 30 to 35 mm. Hg of air through a colon tube or the upper intestinal tract was distended with air through a Rehfuss tube. The eye was carefully examined for ametropic or heterophoric changes and for change in the intraocular tension. No such changes could be detected. We were forced to conclude, therefore, that distention of the colon and the stomach did not

produce visual disturbance by reflex ametropia, heterophoria, or by changes in intra-ocular pressure.

Nevertheless, these experimental procedures produced a *temporary impairment of vision*. How then could it be explained? The optical factors so far not studied were: the amplitude of accommodation, and the condition of the retina itself. It was decided to see if these factors could be influenced by distention of the alimentary tract. The stimulus was applied by Rehfuss tube or in some instances by distending the stomach with a condom balloon tied to a Rehfuss tube. That distention of the stomach by air produces effects similar to those arising from eating a large meal, and, in fact, that the symptoms resulting from eating a large meal are *due* to the distention is evidenced by the similarity of the symptoms. Within a minute or two after distending the gastric balloon the subject became drowsy, felt "relaxed" and desired to sleep, the skin felt warm and moist, perspiration appeared on the forehead, upper lip, palms of the hands, etc., and the knee jerk became markedly diminished. Extra effort was required to read, as the attention lagged and the type became less sharply outlined, that is, somewhat blurred.

The near point was determined, before and after distention, by the various subjective methods. It was found that the accommodation was reduced by from two to five diopters and that a definite near point could not be determined, for the ciliary body reacted so feebly that if the determinations were made within a few seconds of each other the near point would recede by approximately one-half centimeter each time. For example, before distention of the stomach, the near point of the right eye was 7 cm. repeatedly. After distention, the readings taken every 5 to 10 seconds, were 14, 14.4, 14.8, 15, 15.5 cm. After a minute or two the readings were 14, 15, 16, 17, 18, 19, 20 cm. It was certain then that there was a reflex hypotonia of the ciliary muscle causing the near point to recede rapidly with exercise of accommodation. It was, in fact, difficult to determine the near point because the test type or parallel lines used for the test appeared somewhat blurred at whatever distance observed. This could not be easily explained as being due to the hypotonia.

Another factor operating to impair the vision, the retina itself, was next studied.

Examination of the fundus oculi with the large electric ophthalmoscope (Gullstrand) before and after gastric, or gastric plus intestinal distention, showed a striking change in the appearance of the retina and disc, and their superficial vessels. The veins increased markedly in diameter and became considerably more tortuous. Small venules previously not seen came into view. After a period of fifteen to twenty minutes a very definite retinal edema could be observed. The picture resembled a mild case of clinical retinal edema. There was the typical gray white veiling of the

margins of the optic disc and the somewhat hazy appearance of the fundus in general.

SUMMARY

1. Distention of the stomach or intestines in man produces a reflex disturbance in vision. Objects blur, especially near objects. The individual has difficulty in reading, partly due to the blurring and partly due to wandering of the attention.

2. The blurring is not due to ametropia, heterophoria, or change in intra-ocular pressure. It is due to *a*, ciliary muscle hypotonia, i.e., a reflex accommodative insufficiency, and *b*, retinal congestion and edema.

3. The subjective changes (wandering of the attention) are part of the general nervous reaction to the distention. These nervous reactions are similar to those produced by eating a large meal, for the distention initiates the reflexes in both instances. These reactions are: the person feels drowsy, "relaxed" and wishes to sleep, the skin becomes warm and moist (perspiration), and the knee jerk becomes markedly diminished.

STUDIES ON ABSORPTION FROM SEROUS CAVITIES

VI. The Effect of Ligation of the Mediastinal Lymphatics on Absorption from the Peritoneal Cavity

R. S. CUNNINGHAM

From the Department of Anatomy, Vanderbilt University Medical School

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Two principal questions have to be considered in the study of absorption of any type of solution or suspension from the peritoneal cavity. The first of these is the route by which materials escape from the cavity into the circulation, and second the forces involved in effecting this transfer. The various theories concerning both these questions have been recently reviewed (Cunningham, 1926) and need not be given in detail here. In general, however, it has more and more become the accepted opinion that isotonic solutions can be absorbed directly into the blood vessels without the intermediation of the lymphatic system. There have been various theories suggested to explain the absorption of isotonic solutions but this question has not as yet been satisfactorily settled.

Recently Bolton (1921) has returned to the idea that the lymphatic system offers a means of explaining the phenomenon of the absorption of isotonic solutions from the peritoneal cavity. He suggests that, when any solution is introduced into the peritoneal cavity, there is first of all an interchange of water and salts by osmosis and diffusion between the blood vessels and the cavity, and, after isotonicity has been established, the balanced solution is then absorbed via the lymphatics, especially those of the diaphragm. This concept is new in that it brings into play both of the pathways (i.e., blood vessels and lymphatics) postulated by other workers and over which so much discussion has developed in the past. If this conclusion of Bolton's is correct, then any procedure which retards the lymphatic drainage of the peritoneal cavity should obviously delay the rate of absorption of isotonic solutions. The experiments reported here were carried out for the purpose of testing this assumption.

It is well known that the principal route of drainage of the peritoneal cavity, as far as the lymphatic system is concerned, is the elaborate plexus of vessels which is found in the diaphragm. There are two principal groups of efferent trunks draining this plexus. One of these groups passes ventrally to the region of the anterior attachment of the diaphragm and

then runs cephalad along the dorsal surface of the sternum, to end in the anterior mediastinal lymph glands, which in turn are drained by vessels emptying into both right and left thoracic ducts. The other group passes dorsally and drains into the aortic group of glands and thence into the cysterna chyli and the thoracic duct.

When particulate matter is introduced into the peritoneal cavity a large amount of it passes into the plexus of lymphatic vessels in the diaphragm and thence via the anterior group of efferent vessels to the anterior mediastinal lymph glands. A much smaller amount enters the thoracic duct through the dorsal efferent vessels.

In the present series of experiments an effort was made to examine the rates of absorption of isotonic solutions after the mediastinal vessels had been occluded, in order to test whether or not such a procedure would materially affect the rate of absorption. A series of rats, approximately

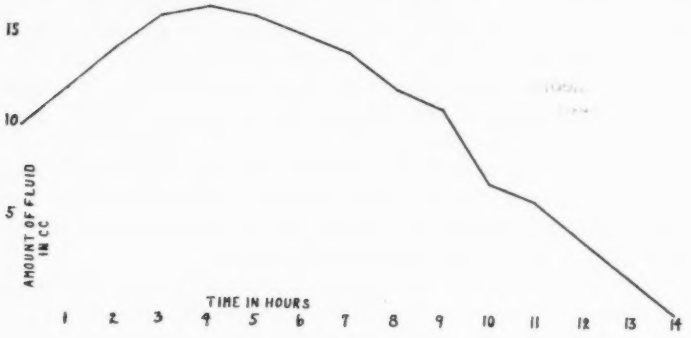

Fig. 1

equal in size, were anesthetized, and, through two small incisions on either side of the sternum a ligature was placed about the entire sternum, drawn very tight and tied. The two incisions were each closed by a single suture and the animals allowed to recover from the anesthetic. Each animal was then given intraperitoneally 10 cc. of a 5 per cent solution of dextrose to which had been added enough Prussian blue to give it a deep blue color. The animals were sacrificed at intervals of one hour and the fluid found in the peritoneal cavity was removed and measured. Figure 1 is a graphic representation of the amounts of fluid recovered from these animals. If this curve be compared with figure 1 of the third paper of this series (Cunningham, 1920), which illustrates the absorption ratios of the same amount of solution by normal rats, it will be seen that the rate of absorption has not been markedly changed from that occurring in the normal animal. A careful examination of the tissues of these animals, in which the

anterior mediastinal lymphatic vessels had been ligated, revealed only a very small amount of Prussian blue; most of this was seen in the liver and spleen, and none was found in the anterior mediastinal glands. When wholly similar amounts of an identical solution, containing Prussian blue, were given to normal rats the amount of dye found in the tissues (liver, spleen and especially the anterior mediastinal glands) at autopsy was very much greater than in the case of the animals of the ligated series. It therefore seems legitimate to conclude that the obstruction of the mediastinal lymphatics brought about a marked diminution in the amount of particulate matter that had been absorbed by the lymphatics of the diaphragm; but that this operation had no appreciable effect on the absorption of the isotonic solution.

There was not quite the same ease in ruling out the participation of the thoracic duct in the process of the absorption of isotonic solutions. Lee (1922) has shown that the thoracic duct can only be effectively ligated in the cat if the entire mass of tissue surrounding the aorta be dissected away and included in the ligature. This procedure was carried out on several rats and intraperitoneal injections of solutions similar to those described above were given. No evidence of any change in the amounts of fluids absorbed was obtained. On the other hand in these animals there was much more absorption of particulate matter than was observed in the animals in which the anterior mediastinal lymphatics had been ligated. In several control experiments attempts were made to inject the lymphatics, after duct ligation had been performed, and in none of these was there any evidence of a patent thoracic duct or functional collateral channels. This, however, must not be considered as conclusive evidence that all absorption via the thoracic duct had been eliminated inasmuch as this operation in the rat may not be as effective, as was shown by Lee to be true for the cat. Since, however, there was greater absorption of particulate matter, as shown by the amount of Prussian blue in the tissues at autopsy, it was not considered necessary to carry out as complete a series as was done with the series of animals in which the anterior group was ligated.

In addition to these experiments a few rats were subjected to both operations, i.e., ligation of the thoracic duct and of the anterior mediastinal vessels, and in these there were still no changes in the rate of absorption of the solution, but, on the other hand, there was even less particulate matter found in the tissues in this series than in either of the other two.

It would thus seem quite probable that the operative procedures described above quite effectively blocked the major portion of the lymphatic drainage of the peritoneal cavity, as indicated by the reduction in the absorption of Prussian blue, but had no appreciable effect upon either the establishing of a balanced solution in the cavity or the absorption of this solution after isotonicity had been reached. These observations would

indicate therefore that an isotonic solution can be absorbed directly into the blood-vascular system without the intermediation of the lymphatics. It must be noted that the solution which was used was not isotonic with the blood, but this was selected both because it allowed the process of the "balancing" of the solution to take place within the experimental animal and because it permitted more accurate comparison with the normal series of controls which had already been established (Cunningham, 1920).

CONCLUSIONS

It has been found that obstruction of the lymphatic drainage of the diaphragm has no effect on the absorption of isotonic solutions from the peritoneal cavity, and therefore indicates that the absorption of such solutions takes place through the blood vascular system.

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"OSCILLATORY VARIATIONS IN THE CONTRACTIONS OF RHYTHMICALLY STIMULATED MUSCLE"—A CORRECTION AND A WARNING

W. B. CANNON AND J. R. PEREIRA[1]

From the Laboratories of Physiology in the Harvard Medical School

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In 1916 Cannon and Gruber published under the title quoted above an account of wave-like variations in the height of the contraction-records of the tibialis anticus muscle of the cat, when subjected to rhythmical and uniform stimulation. Similar waves had previously been recorded by Storey (1904), by Symons (1908) and by Burridge (1910). The periodic oscillations in the records of Cannon and Gruber had all the appearance of being true muscular phenomena: 1. They occurred regularly and persisted for long periods in healthy animals; they were not present or were present for only the brief period of vigorous contraction in animals that were sickly. 2. They could be evoked or abolished by varying the initial tension on the active muscle. 3. Fatigue or a diminished blood supply brought about a decreasing frequency of the waves or their entire elimination. 4. If, because of fatigue, the waves had vanished they could be restored by injecting intravenously adrenalin or by stimulating splanchnic nerves. 5. When the waves were occurring regularly the rate of their occurrence could be increased by splanchnic stimulation, by adrenalin injection, or by lessening the tension on the muscle. 6. The waves were observed after the muscle was curarized; they were not, therefore, of nervous origin.

It seemed interesting to us to learn whether these waves would be different in red and white muscle. Accordingly apparatus was assembled for recording contractions of the tibialis anticus in response to rhythmically repeated stimulations. To our great surprise the phenomena, so common in the earlier experiments, failed to appear, although the conditions for evoking them, mentioned above, were repeatedly reproduced. Comparison of the new with the former experiments showed that the connection of the muscle with the writing lever differed. In the new experiments the muscle tendon was attached by a cord to the vertical side of a rigid triangular metal plate; an extension of the horizontal side of the plate bore the

[1] Fellow of the Rockefeller Foundation.

writing point. The pivot of the lever was at the junction of these two sides. In the former experiments the cord from the tendon passed over a *wheel* and down to a horizontal lever. Fortunately we were able to find the apparatus that was used in 1913 and 1914. On attaching the muscle to it, the waves at once manifested themselves. It did not take us long to demonstrate the occasions for the phenomena. They were as follows:

The wheel proved to be not exactly centered. When the muscle contracted, the wheel was given revolving momentum. Under favorable circumstances, as the muscular tension diminished near the end of a contraction, the turning wheel slipped slightly under the cord. The eccentricity of the wheel, not perceptible on examination, was sufficiently magnified by the writing lever to cause differences in the level of the writing point as the wheel turned. The phenomena, apparently intrinsic in the muscle, can now be explained.

1. The waves occurred and persisted in healthy animals because the contractions were vigorous, and therefore gave to the wheel a quick acceleration which induced slipping. They did not occur in sickly animals because the contractions were too weak to have that effect.

2. They could be evoked by lessening the initial tension and thus allowing the wheel to slip under the cord. They also reappeared when the tension was changed from an after-load to a load—an effect understandable in terms of increased vigor of contraction under the new conditions. If the load (tension) was sufficiently great, however, they failed, because the wheel then could not slip.

3. Fatigue or a diminished blood supply was characterized by a lessening of the energy of contraction. In consequence the wheel no longer slipped, or slipped to a less degree than before when the contractions were quick and extreme. The waves, therefore, vanished or became longer.

4. When adrenalin was injected after the muscle was fatigued, the waves recurred if they had ceased, or were more frequent if they had become slow, because of the invigorating effect of adrenalin, as shown by the higher contractions.

5. The faster rate of recurrence of the waves after adrenalin injections or after the tension on the muscle was lessened can be readily understood from the explanations given above—the wheel slipped to a greater extent than before.

6. The phenomena were seen, to be sure, in a curarized muscle, and therefore were not of nervous origin. But, as shown in the foregoing paragraphs, they were likewise not of muscular origin. *They were due to use of a wheel in the experimental arrangements.*

The artificial character of the "tonus" waves in our experiments led us to look into the conditions which prevailed in other experiments which have yielded similar results. In his stimulating device Storey used revolving

armatures. His apparatus, excepting the magneto, has been placed in our hands. We have found that the construction was defective in that it permitted spread of the current from one sector to another in the disks as the armatures turned. Examination of figure 5 on page 81 of Storey's paper shows that the "tonus" was due to uniform stimulation associated with retarded relaxation. The record reveals that the muscle did not relax smoothly but in little step-like checks which came earlier and more frequently in the regions of the "tonus" rhythms and which consequently prevented prompt lengthening of the muscle in those regions. This effect can be accounted for by very slight stimulations from sparkings between successive sectors after the revolving brush had passed the sector which had given the muscle its main stimulus. Why there should be irregular sequences in the "tonus" waves we have not attempted to learn—the task would involve, we are convinced, an inquiry into the functions of the revolving parts of the apparatus rather than into the functions of muscle.

As further illustration of the danger of wheels we may mention an experience with an ebonite cylinder set with regularly spaced metal sectors and made to revolve under a spring contact. When placed in the primary circuit of an inductorium it produced regularly repeated interruptions of the circuit. A frog gastrocnemius, however, stimulated by the induced current, recorded a regular series of varying contractions recurring with each revolution of the cylinder.

In Symons' experiments on the fatigued gastrocnemius of the frog, which were confirmed by Burridge, the thread attached to the muscle tendon was passed "around the larger wheel of a vulcanite pulley lever." Again suspicions were aroused concerning the rôle of the wheel in the recording arrangements. Symons has informed us, however, that the thread merely passed over the top of the wheel and was fastened to a pin near the under side. His "wave-like variations in the fatigue curves" of the frog's gastrocnemius seemed, therefore, not due to the wheel. This conclusion we have verified by duplicating Symons' records when the muscle was attached directly to a horizontal lever.

That there may be rhythmic oscillations in the height of contraction of cross-striated muscle is proved by the observations of Symons and of Burridge. In a study of the physiological properties of diaphragm muscle Lee, Guenther and Meleney (1916) noted a tendency to "rhythmicity" in its response to stimulation. On using a uniform sequence of shocks, however, the muscle recorded a smooth curve with no sign of rhythmic twitches. They concluded that "most" of the previous rhythmic responses had originated in quantitative irregularities of the shocks supplied by the interrupter, but they still regarded the muscle as having a definite disposition towards rhythmicity. Nice and Neill (1921) likewise have reported oscil-

lations in the contraction of diaphragm muscle and, although they used a pulley in their recording apparatus, Nice has informed us that he has observed the waves when the pulley was not employed. In figure 6 of the paper by Cannon and Gruber there were what appear to be two sets of waves—a phenomenon difficult to explain as the result of the single wheel. The possibility of oscillations in the vigor of contraction of skeletal muscle may be admitted, and we had intended to pursue the question further; other interests have prevented us from doing so. We are inclined to be highly suspicious of the physiological origin of these oscillations. In any event the experiences above recorded should be useful as a warning to *beware of wheels* in apparatus for stimulating or recording.

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THE "PHYSIOLOGICAL MAXIMUM HEART RATE" AS AN ARTEFACT

W. B. CANNON AND J. T. LEWIS[1]

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Hooker (1907) appears to have been the first to employ the phrase "physiological maximum rate of the heart"—defined as a rate "which is usually reached after section of the vagi and beyond which it can only with difficulty be increased." The concept is based on observations by a series of investigators, beginning with Roy and Adami (1892). They reported that with one or both vagi intact direct or reflex excitation of the augmentor nerves is always accompanied by acceleration of the heart, greater the slower the beat, but that after section of both vago-sympathetics in the neck the same stimuli do not increase the rate. Dogs were studied—under chloroform, ether or morphia, singly or combined. MacWilliam (1893), making use of the cat anesthetized with chloroform, found that after section of the vagi and while the cardiac augmentor nerves remained undisturbed the heart beats at a "high maximum rate" (216 to 250 per minute) and that either reflex or direct stimulation of the augmentor nerves does not cause an appreciably faster beat. Direct stimulation did accelerate the heart if after vagus section the pulse became slow because of continued low blood pressure. Chloroform alone, according to MacWilliam, causes the heart to beat more slowly after all connecting nerves have been severed—an effect which he interpreted as a depressant influence exerted on the intrinsic rhythmic mechanism of the organ. The ineffectiveness of the accelerators after removal of tonic vagus inhibition was further emphasized by Hunt (1899), who experimented chiefly on dogs which were anesthetized commonly with morphine and ether. Although he had noted earlier (1897) effects from direct stimulation of the accelerators after the vagi had been cut, he found no reflex acceleration. Furthermore, both MacWilliam and Hunt were unable to accelerate the beat reflexly when the rapid pulse, after double vagotomy, was moderated by stimulating a peripheral vagus trunk.

The difficulty of obtaining any reflex increase of pulse frequency in vagotomized dogs or rabbits under anesthesia was confirmed by Hooker

[1] Fellow of the Rockefeller Foundation.

(1907). He did record, however, a reflex acceleration which could be induced if the heart was made to beat much more slowly than usual (as slowly as 24 to 6 beats per minute in all cases except two) by exciting the peripheral vagus trunk. He explained the failure of Hunt and MacWilliam to obtain this result by assuming that they did not sufficiently reduce the rate, and he suggested that perhaps the accelerator mechanism becomes progressively more irritable as the consequences of a slow pulse make it important for acceleration to occur. Bainbridge (1914) likewise was able to evoke a faster heart rate, in dogs under morphia *plus* ether and chloroform, after eliminating vagus influences and then checking the heart, either by peripheral vagal stimulation or by giving pilocarpine or by taking advantage of the naturally moderate beat which occasionally prevails. In the last condition he found that the acceleration induced reflexly tended to persist and that if the stimuli were repeated a "maximal" rate was established, not raised further by injection of adrenalin (1 cc. of 0.05 per cent). Bainbridge was first to consider that reflex secretion of adrenin might play a rôle in accelerating the heart, but he was able to demonstrate that the phenomena noted by him occurred after the adrenal glands had been excluded.

Most of the experiments described above have recently been repeated by Tulgan (1924a, b). Using cats under ether he observed 1, no reflex acceleration after section of only the inhibitory nerves of the heart (repetition of the experiments of Roy and Adami, of MacWilliam, of Hunt, of Hooker and of Bainbridge); 2, reflex acceleration if, under these standard circumstances, vagal slowing was induced (repetition of the experiments of Hooker, and of Bainbridge); and 3, failure of acceleration if, under the standard circumstances, a "small amount of adrenalin" (concentration, dose and rate of injection not reported) is introduced into the femoral vein (repetition of Bainbridge's experiment). From these results Tulgan laid down the doctrine that "after both vagi have been sectioned the rate of the heart is at its physiological maximum and it cannot be increased any further by stimulation of a sensory nerve or by the injection of adrenalin" (a condition suggested by MacWilliam and by Hooker, but not stated so absolutely by them).

In 1925 Cannon and Britton reported 15 experiments in which the maximal rate of the denervated heart varied between 202 and 308 beats per minute; in 13 of the 15 the maximal rate was 230 or faster. These animals were in an *unanesthetized*, pseudaffective state. Cannon and Britton called attention to the fact that in the figures Tulgan published the maximum heart rate for the cat was set down as 210 beats per minute; and on the basis of the 15 experiments cited above they remarked that 210 was not physiological maximum "in the sense that the heart is unable to beat faster than 210." Indeed, in 10 of the 15 cases of their series the heart

rate during quiet was above 210 (averaging 239), and it rose during activity to an average of 261. These figures, high compared with Tulgan's, naturally led to questions. How might the difference be accounted for? Was it due to a difference between the anesthetized and unanesthetized condition? Might ether limit the ability of the heart to respond to accelerator stimulation?

In answer Tulgan (1926) has urged that the completely denervated heart beats more slowly than the heart with nerves intact or with vagi cut; that Cannon and Britton, not having compared the rates under these varying conditions, could not be sure that the increases observed actually lifted the rate "above the physiological maximum;" and that they did not give sufficient statistical evidence to support their suggestion. Although the completely denervated heart may beat more slowly than the heart with normal or accelerator innervation, in 13 of our 15 cases of complete denervation in the *unanesthetized* pseudaffective state with adrenals active the maximal rate was *much faster* than that reported by Tulgan for *anesthetized* animals with accelerators and adrenals active. And although the high rate in our animals may not have been above their "physiological maximum," it was far above the physiological maximum in Tulgan's records. Again the question arises as to the reason for the difference.

In taking up the suggestion that anesthesia might account for the difference Tulgan performed experiments under *light* ether (with faster heart rates than he has previously reported), and observed that the rate after dividing the vagi was higher before decerebration than it was afterwards with ether removed. In every one of the experiments in which the vagotomized animal was completely decerebrated and then freed from the light ether the heart rate fell—in one instance as much as 44 beats per minute, from 240 to 196. These results show that under the circumstances of Tulgan's experiments light ether had no marked depressant effect. But they bring out a significant point which Tulgan seems not to have regarded. The heart in the decerebrated animal was still innervated by accelerators. But according to Tulgan the heart thus innervated beats at its physiological maximum. In the case just cited if 240 was the maximal rate, was 196 also a maximal rate, as the "law demands?" Could the rate have been raised above 196 by afferent stimulation or adrenalin? Unfortunately Tulgan did not make these simple tests. It is clear, however, that in spite of the presence of accelerator fibers the rate may vary greatly under different circumstances. The "law," therefore, must be limited and conditioned if it is to correspond to the observed facts.

In an investigation directed towards having a completely denervated heart in surviving animals Cannon, Lewis and Britton (1926) encountered much difficulty because of the presence of accelerator fibers passing to the heart from the thoracic sympathetic chain below the stellate ganglia.

The vagus fibers had been eliminated. The conditions, therefore, were such as those described above in the experiments of Roy and Adami and their successors. The behavior of the heart under these circumstances is significant in relation to the so-called "physiological maximum."

As shown in table 3 and in figure 3 of the article by Cannon, Lewis and Britton, healthy, unanesthetized animals, deprived of humoral accelerators but with nervous accelerators present and with vagi cut, do not exhibit a maximal heart rate, or anything near it, so long as they remain quiet. In 21 observations on 4 animals under those conditions the average heart rate was 146 beats per minute. When the animals became excited and active, however, the rate promptly rose. In these 21 observations the average increase was from 146 to 198, an increment of 52. In some instances the pulsations were faster by 70, 80—indeed, by more than 100 beats per minute. And these remarkable increases almost wholly disappeared when the accelerator fibers were excised; in 21 observations on the same 4 animals after exclusion of accelerator impulses the average rise of rate caused by vigorous struggle was from 136 to 145—a change of 9 beats per minute instead of 52. These results are concordant with observations made in 1914 by Gasser and Meek. They noted in two instances that exercise caused the heart of the vagotomized and adrenalectomized dog to pulsate faster by 24 beats per minute (160 to 184), and they thus showed that the accelerators may act in spite of being the only cardiac nerves. All this evidence yields no support for the dictum that when only nervous accelerator influence is present the heart beats at its physiological maximum rate and that it cannot be made to beat more rapidly. Not only does it not beat at its maximal rate, but the rate can be strikingly increased.

The condition is different under anesthesia. In such animals as those just described, the alterations induced by etherization are shown in the following protocols:

Cat, female, 2.4 k. May 19, 1925, right vagus cut above heart in thorax, left vagal cardiac fibers severed; both stellate ganglia removed. May 28, right adrenal removed, left denervated; liver nerves cut. June 11, h. r. (heart rate), when cat quiet on lap, 132; increased by struggle on board to 236, a rise of 104 beats per minute. June 12, cervical sympathetic strands removed; vagi tested and proved without action on heart. June 29, 9:24, h. r., cat quiet on lap, 136; 9:28, on holder after struggle, 257—a rise of 121 beats per minute. At 10:29, etherization begun; 10:35, on holder under ether, h. r. 187. Sciatic nerve stimulated (secondary coil Harvard inductorium, 8 cm., 30°) through electrodes inserted through skin (leg muscles tetanized), maximal h. r. 199—a rise of 12 beats. At 10:55, h. r. 171; stronger stimulation of sciatic (coil, 7 cm., 0°), maximal h. r. 178—a rise of 7 beats. Electrodes introduced in front of first dorsal vertebra and back of fourth dorsal, and cord stimulated (at 11:10); forced extension of fore limbs; h. r. increased from 163 to 172—a rise of 9 beats per minute. H. r. gradually slower; at 11:21, 156 beats per minute. At 11:25, ether stopped, h. r. 160; 11:35, movements, h. r. 172; 11:40, tossing on board,

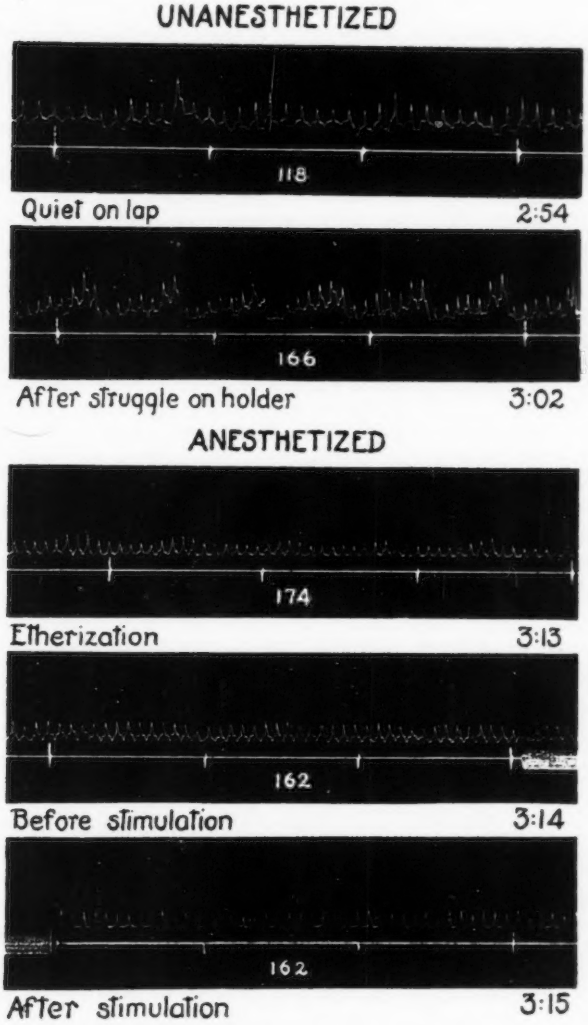

Fig. 1. Record of heart beats of an animal with humoral accelerators excluded, with nervous accelerators active and with vagi severed; time recorded in 5-second intervals. *2:54*, cat quiet on lap, heart rate 118 beats per minute. *3:02*, cat in holder and after struggle; heart rate 166. *3:13*, cat on holder and under ether anesthesia (administration of ether begun at *3:04*); heart rate 174. *3:14*, at start of strong stimulation of the right sciatic nerve, heart rate 162. *3:15*, at end of 30 seconds of stimulation of the sciatic nerve, heart rate 162.

h. r. 186; 11:45, same, h. r. 196; 11:58, struggle, h. r. 212; 12:07, quiet on board, h. r. 184; after slight struggle, h. r. 208—a rise of 24 beats. At 12:21, when cat quiet on lap, h. r. 138—a drop of 74 beats per minute. July 1, struggle increased h. r. from 128 to 236—a rise of 108.

Cat, male, 2.8 k. Dec. 18, 1926, right vagus and common cardiac nerve cut above heart in thorax; cardiac branches of left vagus severed. Thoracic sympathetic chains removed third to ninth rib on both sides; stellate ganglia not disturbed. Right adrenalectomy, left abdominal sympathetic chain removed from diaphragm to kidney, left splanchnics cut. Hepatic nerves divided. Jan. 12, 1927, right abdominal sympathetic chain taken out as on the left side, right splanchnics divided. Jan. 26, left adrenal medulla sucked out. Jan. 31, a quick rise of 76 beats (96 to 172), after struggle on holder, indicated that nerves had reconnected with the heart. Jan. 31, an increase of 38 beats on struggle, after atropine sulphate (2 mgm. intravenously) effective, as proved by the rigid iris. Feb. 11, h. r., quiet on lap, 120 beats per minute. After struggle on holder, h. r. 166—an increase of 46 beats. Ether was now given, and the rate rose to 174; as the anesthesia deepened the rate fell to 162. The right sciatic nerve was now stimulated for 30 seconds (secondary coil, 8 cm., 0°) *via* electrodes thrust through the skin (leg muscles tetanized); h. r. 162 beats per minute, no change. (The original records are shown in fig. 1.)

From the foregoing observations it is clear that the capacity of the heart to respond to accelerator stimulation is very different in the *anesthetized* as contrasted with the *unanesthetized* animal. The results reported in these protocols were confirmed in other instances. For example, a cat with heart denervated by stellate removal and vagus section, with right adrenal gland, upper abdominal sympathetic chains, splanchnics and semilunar ganglia all removed, and with the medulla of the left adrenal sucked out, showed after four weeks a prompt rise of 106 beats (from 152 to 258) on struggling. The absence of humoral accelerators and the quickness of the effect both indicated a nervous cause of the acceleration. The basal rate (152) was too high to permit the faster beat to be accounted for as inhibition of vagus tone. It must be due to accessory accelerator fibers. The animal was etherized. Thereupon the rate ranged between 248 and 256 beats per minute. The chest was now opened in the fifth intercostal space on the right side and the sympathetic chain was dissected out from the 8th rib upward to the end at the 3rd rib. Immediately the heart rate dropped to 144 beats per minute. Two hours later it had fallen further to 120. Obviously etherization had resulted in forcing the heart rate almost to the maximal level, where a further increase would be almost impossible. The very fast pulse was caused by accelerator impulses from the thoracic sympathetic chain, for it promptly ceased when the chain was excised. In another instance, in which cardiac denervation had been performed without disturbing the stellates, and the accelerator fibers had regrown, the heart rate, after struggle, increased 38 beats per minute though all nervous control of humoral agents had been eliminated. Etherization increased the rate first from 112 to 160 and later dropped it to 140. Very

strong stimulation of the sciatic nerve (secondary coil, 4 cm.), attended by marked tetanization of the leg muscles and labored respiration, caused a maximal increase of 12 beats per minute. In all these instances of accelerator action in the absence of vagal influence, marked variations of heart rate occurred in the unanesthetized state. When anesthesia was induced, however, the rate rose above the basal resting level, but not to the maximal degree, and thereafter it could be increased only slightly in spite of powerful stimulation of sensory nerves or, in one instance, stimulation of the spinal cord in the region supplying cardio-accelerator nerves.

All these observations taken together lead us to conclude that the so-called "law" of the physiological maximum heart rate, reported as typical of the heart supplied only with accelerator fibers, is a phenomenon due to anesthesia. How it may be accounted for we do not attempt to decide. There are facts which suggest that it may result from two conditions: 1, a stimulating, later a depressant influence of the anesthetic, reducing the ability of the heart to respond to sympathetic impulses at the true maximal rate; and 2, a discharge of sympathetic impulses under anesthesia so that the heart is driven at or near the limit of its reduced ability to respond. This hint accords with the observation that the heart, still provided with accelerator fibers, beats more rapidly under anesthesia than it does at its basal resting level without anesthesia, and yet does not have its maximal pace and is not made to assume it by artificial stimulation.

SUMMARY

The evidence is reviewed which has led to the conclusion that the heart innervated only by accelerator fibers beats at its physiological maximum rate, which cannot be increased reflexly or by adrenalin injections.

In normal *unanesthetized* animals, however, with accelerator influence alone active, the heart rate varies greatly as they change from rest and calm to activity and excitement. In such animals *anesthesia* increases the rate to a point below the maximal and there fixes it, so that reflex and direct stimulations have little or no influence (see fig. 1).

The so-called "law" of the "physiological maximum rate of the heart" must be regarded, therefore, as descriptive of an artificial state induced by the anesthetic agent.

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STUDIES ON THE KNEE JERK

I. A Simple, Dependable and Portable Knee Jerk Apparatus for Use on Higher Mammals and Man[1]

CARL A. JOHNSON

From the Physiological Department of the University of West Virginia and the Hull Physiological Laboratories of the University of Chicago

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From experience with the various types of knee jerk apparatus used in teaching and research, and from a study of the description of others as found in the literature, it was evident that each had its limitations in point of applicability to quantitative investigations. Among these may be cited the apparatus lately devised and used by Tuttle (1924) for investigations on human subjects. This apparatus, although it incorporates several new features, is essentially a modification of an apparatus used at the University of Chicago for some twelve to fifteen years. Even though the apparatus used by Tuttle has added improvements, it is not, perhaps, as simple in construction, and as practical, as the one about to be described.

There are several factors of prime importance which must be considered in working with the knee jerk, and these I shall list without giving them further consideration at this time:

1. The stimulus to the patellar tendon must remain constant.
2. The hammer must always strike the same place.
3. The interval between stimuli must remain constant.
4. There must be no "after fling" which would give more than one stimulus.
5. The apparatus should be applicable to lower forms, such as dogs and cats.
6. The apparatus should be portable.

With these considerations in mind, the construction resolved itself into distinct parts, and these I shall enumerate and consider separately in detail:

1. The stimulating device (fig. 1, *A*).
2. The device for initiating the stimulus (fig. 1, *B*, *C*, *D*, *E*, *F*).
3. The recording device (fig. 2).

[1] Aided by a grant from the Billings Medical Club Research Fund, Chicago, Ill.

4. The device for holding the leg in the same position throughout the experiment (fig. 3).

1. *The stimulating device:* Since the laboratories of the University of Chicago are equipped with 110 volt, direct current, and this is maintained rather constant, it was thought desirable to use this as a source of energy.

A brass tube 11 inches long and $\frac{3}{8}$ inch inside diameter, was procured (see fig. 1, *A*, *5*) and upon one end a brass disc, $3\frac{1}{2}$ inches in diameter and $\frac{1}{8}$ inch wide, was fastened with the hole in the tube patent (fig. 1, *A*, *4*). Another brass disc was slipped over the other end of the tube for a distance of 4 inches (fig. 1, *A*, *4*). Intervening between the two discs, copper wire (three pounds of no. 18 cotton covered wire) was wound. This makes the dimensions of the coil $6\frac{3}{4}$ inches long and 2 inches in diameter (fig. 1, *A*, *9*). The two free ends of this wire were left long enough for later connections.

It is probably understood that upon one end of this arrangement there is a free end of brass tube, 4 inches long (fig. 1, *A*, *5*). On each side of this tube a longitudinal slot $\frac{1}{8}$ inch wide and $2\frac{3}{4}$ inches long is so made that the slot stops short $\frac{1}{2}$ inch from the end of the tube (fig. 1, *A*, *7*).

A soft iron rod (fig. 1, *A*, *3*) $8\frac{1}{2}$ inches long and of a diameter slightly less than the inside diameter of the brass tube, is suitably joined to a brass rod 4 inches long and of the same diameter (fig. 1, *A*, *2*). This rod can then move freely within the brass tube. Two cotter pins are fitted transversly through the iron end of the rod, the first, (fig. 1, *A 6*), $1\frac{1}{4}$ inch from the end, and the second (fig. 1, *A*, *6*) $2\frac{3}{4}$ inches from the end. This allows a longitudinal movement of the rod of 1 inch (the position of the second pin can be altered, so as to give greater movement if so desired). On the brass end of the rod a hard rubber knife edge hammer is fastened (fig. 1, *A*, *1*). A spring (fig. 1, *A*, *8*) of sufficient strength surrounds the brass tube, one end pushing against the second pin (fig. 1, *A*, *6*), and the other pushing against the brass disc (fig. 1, *A*, *4*).

The whole mechanism is mounted in such a manner as to give both vertical and longitudinal adjustments of position. This is not illustrated in the diagram, but the photograph (fig. 3) shows the mounting.

If there is no force acting upon the iron rod, it will remain in the position as indicated in figure 1, *A*. If an electric current is sent through the wire coil, the iron rod will be pulled forward in the tube against the tension of the spring. The force of the movement can be varied by varying the amount of resistance of a 50 ohm rheostat (fig. 1, *F*).

The interval during which the hammer rests upon the patellar tendon must obviously be very short in order not to modify the results by pressure and other effects. To illustrate this point, one need only consider the number of stimuli delivered during the course of one experiment. In one of the acute experiments, using a dog under light barbital anesthesia,

approximately six thousand blows were delivered to the patellar tendon during the interval of the experiment which lasted for seventeen hours. To make the hammer return immediately after striking the tendon, the time interval for the passage of the current must be very short. This point will be considered again in the description of the initiating device.

2. *The device for initiating the stimulus:* This device consists of three

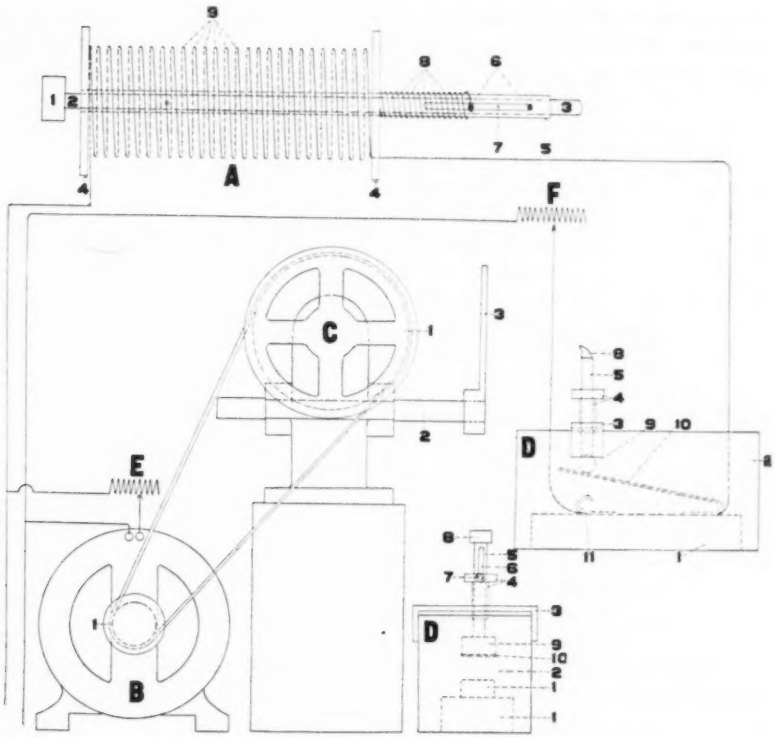


Fig. 1. Illustrates diagrammatically the stimulating device and the initiating device, the details of which are given in the text. Scale, 1:4.

parts: a, an electric motor (fig. 1, *B*); b, a speed reducer (fig. 1, *C*); c, and a switch operated from a cam on the speed reducer (fig. 1, *D*).

The electric motor is a small direct current motor of the Westinghouse type ($\frac{1}{20}$ H. P. with 1750 R. P. M.). This is furnished with a 1-inch pulley (fig. 1, *B*, *I*) which drives a $3\frac{5}{8}$ inch pulley on the speed reducer (fig. 1, *C*, *I*). This in turn drives the worm gears which are so cut as to

give a speed reduction of forty-eight to one (Boston Gear Co., Speed Reducer B U-2, 48:I). The total speed reduction from the pulleys and the gear arrangement is then forty-eight times three and five-eighths, or approximately one hundred and seventy-four to one. A further speed reduction is possible by means of a five hundred ohm resistance (fig. 1, *E*—Vitrohm Variable Resistance—procured by the Westburg Engineering Co.) which is interposed in series with the motor and the line. By varying the resistance *E*, the interval between stimuli may be varied from 6 to 10 seconds. Further changes in the interval may be had by altering the ratio between the pulleys (fig. 1, *B*, *I* and fig. 1, *C*, *I*).

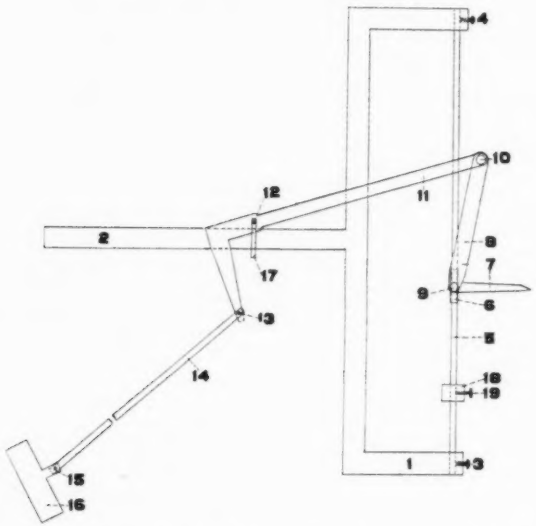


Fig. 2. Shows the construction of the recording device, the details of which are given in the text. Scale, 1:4.

By rotation of the pulley (fig. 1, *C*, *I*), the worm gears, inside of the metal case of the speed reducer, drive the shaft (fig. 1, *C*, *2*). Upon the end of the shaft is fastened rigidly a cam (fig. 1, *C*, *3*) which operates the switch (fig. 1, *D*).

The cam (fig. 1, *C*, *3*) was made long in order to facilitate the adjustment of the time during which the current is allowed to flow. In rotating it is obvious that the cam will describe a circle, the circumference of which is determined by the length of the cam. Therefore the distance covered by the end of the cam is, in reality, a function of the length of the cam—the cam, in other words, constitutes the radius of the circle described.

By this arrangement it is possible to apply greater pressure upon the switch contacts and still maintain a very short interval for the passage of the current.

For the sake of simplifying the description of the switch a longitudinal as well as a transverse section was drawn. The switch proper is mounted in a metal box which contains castor oil. Running the switch in oil was found necessary to reduce the amount of sparking and thereby preserve the contacts of the switch. The two contacts are mounted upon a piece of slate (fig. 1, *D*, *1*), and this in turn is fastened rigidly to the bottom of the metal container (fig. 1, *D*, *2*). Upon the top of the container (fig. 1, *D*, *2*) is mounted permanently a piece of strip iron (fig. 1, *D*, *3*) in the position indicated in the drawing. Upon this is mounted a metal sleeve (fig. 1, *D*, *4*) in which a metal tube (fig. 1, *D*, *5*) can slide freely. In the metal tube (fig. 1, *D*, *5*) is made a slot (fig. 1, *D*, *6*) in order that a machine screw (fig. 1, *D*, *7*) mounted on the metal sleeve (fig. *1*, *D*, *4*) may limit the upward and downward movement of the tube. Upon one end of the tube (fig. 1, *D*, *5*) is mounted a section of a cylindrical rod (fig. 1, *D*, *8*). The other end of the tube carries a cylindrical rod (fig. *1*, *D*, *9*) in the position indicated in the drawing. The switch contacts (fig. 1, *D*, *10*, *11*) are made of spring nickel.

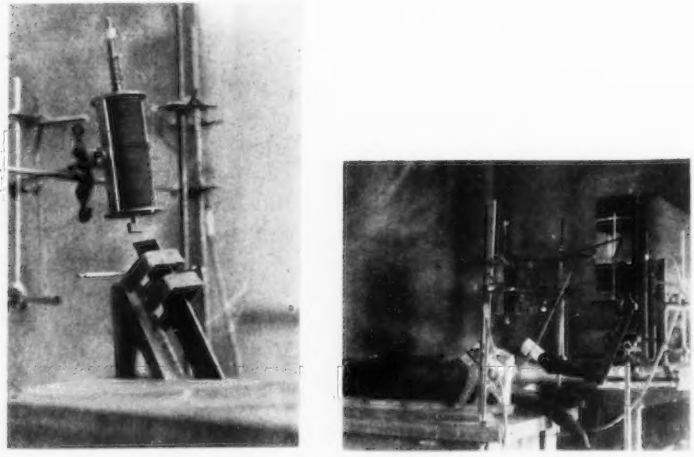
For each rotation of the cam (fig. 1, *C*, *3*) there is a downward movement of the switch contact (fig. 1, *D*, *10*) and the circuit is made through contact (fig. 1, *D*, *11*).

The wiring diagram as illustrated is diagrammatic. A 50-ohm rheostat (fig. 1, *F*) is connected in series with the line, the switch and the stimulating device. By means of this resistance it is possible to get all ranges of blows from the weakest to the strongest blow, or the blow the instrument would give if connected directly with the hundred and ten volt line.

3. *The recording device:* There are several points to consider in the construction of a device which will record the knee jerk as a result of a blow to the patellar tendon: *a*, the record should show a vertical line; *b*, there should be no lost motion; *c*, the record should be an accurate index of the extent of the jerk; *d*, the instrument should be easy to set up; *e*, there should be adjustments for maintaining an even base line; *f*, and the parts should be interchangeable such that the actual response can be amplified or depressed upon the record.

With these considerations in view, the device as illustrated in figure 2 was constructed. It is essentially a modification of a Keith-Lucas recording apparatus. *1* and *2* represent a rigid metal frame upon which the various movable and non movable parts are mounted; *5* is a steel drill rod which can be removed by loosening set screws *3* and *4*; *6* is a metal sleeve which moves in a vertical plane upon the steel drill rod; *7* is a pointer which is fastened rigidly to *6*; *8* is strip of bronze which is fas-

tened to *6* and *11* by movable joints *9* and *10; 11* is a strip of bronze which moves on the fulcrum *12* and is fastened to *8* and *14* by movable joints *10* and *13*, *14* is a steel drill rod which is fastened to *11* and *16* by movable joints *13* and *15; 16* is a cuff which is fastened to the dog's leg; *17* is a brass clamp which carries the fulcrum, *12*, and can be moved along the rod by means of a set screw not shown in the diagram; *18* is an adjustable collar which can be held at any part of the drill rod, *5*, by means of set screw, *19*. It limits the downward movement of the sleeve, *6*, carrying the writing point *7*, and insures a straight base line.



1 *2*

Fig. 3. *1* illustrates the electromagnetic hammer mounted in *position* above the device designed to hold the leg in a fixed position, so that the hammer always strikes the patellar ligament in the same spot.

2 shows a deeply anesthetised dog with the leg in the holder. Above the knee is seen the electromagnetic hammer. The recording device attached to the leg is shown registering a record on a smoked surface.

Constructed primarily for use on the dog, the recording device can be adapted readily for recording the knee jerk of the human being by introducing a longer drill rod, *14*, and bronze strip, *11*, which is suitable for the proper propagation of the thrust.

4. *The device for holding the leg in position throughout the experiment:* For work on man there are many chairs which have been described in the literature, and therefore we feel it unnecessary at this time to go into detail of the one used by us, although this will be published in a later communication.

Since a great number of the experiments are acute and are done on lower animals, especially the dog, a brief consideration of the holder will be given at this time.

The holder must obviously be made to fit the animal to be used. The one used by us was made to accommodate dogs weighing from 15 to 20 kilos, although we now have an additional holder to be used with cats.

The dog is laid upon a dog board of the ordinary laboratory type. At first is was thought necessary to tie the front legs, but now satisfactory results are had by merely laying the dog on the side opposite the leg upon which the knee jerk is to be elicited. A small board of the width of the hind leg and of a length equal to the distance from the dog board to the popliteal fossa of the hind leg is fastened to the dog board at an angle of

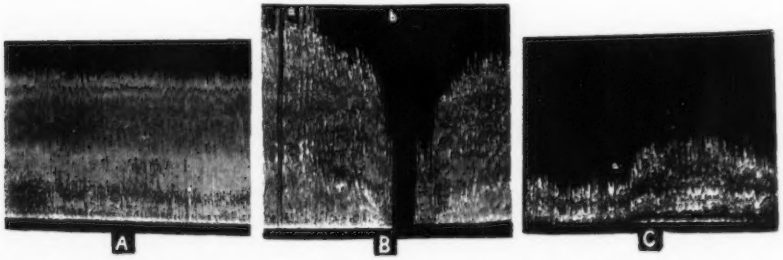


Fig. 4. Tracing showing the character of the knee jerk in the anesthetised dog under various experimental conditions.

A = Showing the character of the response in a dog under barbital-Na anesthesia with the blow to the tendon at 7-second intervals.

B = Illustrates the quantitative diminution and increase in the knee jerk as a result of administration of ether by inhalation, *a*, and its withdrawal at *b* with the subsequent slow return of the knee jerk to the normal level.

C = Illustrates the increase in height of the knee jerk as a result of the intravenous injection at *a* of 1 mgm. of strychnine sulfate.

105 degrees with the horizontal. The ham string muscles rest upon this small board (fig. 3).

In the early experiments an incision was made in the region of the ham strings and the femur was exposed. This was securely wired to the board. This type of procedure gave fairly good results, but it was thought advisable to try several methods, for it seemed that trauma might influence the results.

It was found unnecessary to fasten the femur directly by any method, and, as the experiments are now carried on, the leg is held in position upon the small board by means of strip iron clamps which are made to fit the leg snugly, and still not interfere with the blood supply (fig. 3).

For purposes of illustration, portions of tracings, taken during experi-

ments, are shown in figure 4. These illustrate the nature of the knee jerk in a barbitalised dog during a normal period, a period of depression of the cord, and during a period of excitation of the spinal cord.

Figure 4, *A*, represents a portion of a normal tracing in which the interval between stimuli was seven seconds. It might seem that this interval is rather short when one considers that a single experiment may last for seventeen continuous hours, but in our experience there appeared to be no deleterious effects from stimuli repeated at this interval. As far as edema at the point of impact and fatigue from the repeated stimuli are concerned, there is no apparent marked effect. At the end of the experiment after various procedures, the jerk appeared, as far as we could determine, almost as high as when we started. Incidentally, the experiment lasting for seventeen hours was the longest of the series, but it would have been possible to continue for a much longer period if it had seemed advisable.

Figure 4, *B*, shows the effect of ether on the knee jerk when given by the inhalation method. This tracing was inserted, not as evidence that ether depresses the spinal cord, but merely to show the diagrammatic fall in the knee jerk as the concentration of ether in the tissues increases and the diagrammatic return of the jerk as the concentration of ether in the tissues becomes less.

Figure 4, *C*, illustrates the change in the height of the knee jerk from stimulation of the spinal cord by means of strychnine. By this means it has often been possible to raise the irritability of the cord when it had been depressed by various means, or it has been used in comparison with other drugs when there has been some question as to the action of the particular drug. We felt justified in using strychnine as a criterion of cord stimulation, since it has been commonly conceded by pharmocologists to be a cord excitant, and our results have shown nothing to the contrary.

SUMMARY

The apparatus and procedure as set forth in this article have many advantages. The apparatus is simple and convenient to operate, it is automatic, it can be run for long intervals, and has adjustments as to the strength of blow and the interval between blows, and is readily applicable to man and to the common laboratory animals, such as the dog and the cat.

The advantages of doing preliminary work on lower forms are obvious. The long normals which can be taken and the great number of responses give one more justification for drawing conclusions than if only a few knee jerks are taken. It has been our experience (at least in working with anesthetised and unanesthetised dogs and also in working with unanesthetised humans) that the knee jerk is modified by many factors confirming

earlier work on the subject, particularly that of Lombard (1887), and hence the jerk is very irregular, especially in unanesthetised animals and humans. From this it seems only logical to conclude that interpretations of results must be made from a great number of jerks and the general trend of the jerks taken over a long period of time.

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THE RÔLE OF THE HYPOPHYSIS IN THE INITIATION OF LABOR

H. B. VAN DYKE AND ADOLPH KRAFT

From the Department of Physiological Chemistry and Pharmacology, University of Chicago, and the Obstetrical Division of Cook County Hospital, Chicago, Illinois

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Since the suggestion of Herring (1908) that at least one of the pathways of secretion by the posterior lobe of the hypophysis is into the cerebrospinal fluid of the third ventricle a considerable amount of contradictory evidence as to the presence of the "hormone" in the *liquor cerebrospinalis* has accumulated.[1] Recently Dixon (1923) pointed out that dog cerebrospinal fluid has all of the characteristics of a dilute extract of the *pars posterior* and that the concentration of this substance may be increased by the intravenous injection of ovarian extract but not by similar injections of corpus luteum or other tissue extracts. Dixon and Marshall (1924) later performed experiments which led them to conclude that changes in ovarian secretion can cause an increased secretion by the posterior lobe into the cerebrospinal fluid and that this may be an important factor in the initiation of labor. Extracts of sow ovaries of animals at or near term increased the concentration of oxytocic substance in the cerebrospinal fluid of dogs; such an effect was not seen when ovarian extracts of animals in earlier stages of pregnancy were used. Recently, however, Blau and Hancher (1926) offered evidence which throws much doubt on the specificity of the presumed "stimulation" of the pituitary gland by ovarian extracts. These workers found that the oxytocic substance of cerebrospinal fluid could be greatly increased not only by ovarian extracts but also by extracts of the liver, testis and spleen.

We have attempted to test this theory experimentally in a somewhat different fashion by titrating the concentration of oxytocic substance in cerebrospinal fluids withdrawn from women before and during labor. With data so secured the present paper is concerned.

TECHNIC. In each of the cases[2] reported about 5 cc. of cerebrospinal fluid were removed by lumbar puncture performed in the usual manner. In no case was this procedure followed by untoward effects. Only speci-

[1] The literature is reviewed by Miura (1925).

[2] Patients for this study were made available through the kindness of the late Dr. W. G. Lee. Dr. T. J. Morris obtained three of the specimens for us.

mens which macroscopically appeared absolutely blood-free were tested. To ensure as little loss of oxytocic substance as possible either solid NaH_2PO_4 (to make a M/20 solution) or acetic acid (to make a M/240 solution) was added to the ampoules at the time of filling. The ampoules were kept on ice after being sealed and sterilized in boiling water. Control experiments with very dilute posterior lobe extract indicated that such treatment was safe for the few days which necessarily elapsed between the collection and the testing of the specimens.

Except for control blood pressure experiments on the cat to ascertain whether or not histamine-like bodies might be present, all experiments were performed on the isolated virgin guinea-pig uterus attached to a lightly weighted isotonic lever and suspended in the usual manner in a 50 cc.

TABLE 1

The concentration of oxytocic substance in the cerebrospinal fluids of pregnant women

NUMBER	PATIENT	PREGNANCY	IN LABOR	CEREBROSPINAL FLUID	OXYTOCIC SUBSTANCE PRESENT (+) OR ABSENT (−)	MINIMUM CONCENTRATION OF FRESH P. POSTERIOR CAPABLE OF EXCITING UTERUS	ESTIMATED CONCENTRATION OF OXYTOCIC SUBSTANCE IN CEREBROSPINAL FLUID
			days later	*cc.*		$\times 10^{-4}$ *mgm. per cc.*	$\times 10^{-4}$ *mgm. per cc.*
1	IH	2	19*	4.1	−	0.5	<6
2	MA	2	0.5	3.6	−	0.5	<5
3	MB	1	25	4.1	−	0.5	<6
4	MJ	1	1	3.5	+	1.0	14
5	RK	1	16	4.4	+	0.5	6
6	JW	2	19	4.2	+	0.5	6
7	FC	1	15	4.0	+	1.0	12
8	CC	2	0.5	5.0 } 10.0	+	1.0	5
9	AG	3	30	5.0 }			

* Patient had false labor pains for 4 hours at time spinal puncture was done.

bath containing Tyrode's solution. We used as a quantitative standard an extract of the international standard posterior lobe powder kindly given us by Professor Voegtlin. One milligram of the dry powder was considered equivalent to 7 mgm. of fresh posterior lobe. The bath used was fairly large; so *relatively* insensitive uteri were subjected to repeated stimulation by pituitary extract during which the sensitivity more or less quickly mounted. Then, not infrequently, a slight but definite contraction could be elicited by one part of fresh posterior lobe in 20,000,000 parts of Tyrode's solution. Quantitative data are of course difficult to secure under such circumstances; the data given in the tables usually represent only approximate quantities.

Immediately before being added to the uterine bath the cerebrospinal fluid was carefully neutralized with isotonic NaOH to a pH of about 7.4

(phenol red). When the fluid had been acidified by NaH_2PO_4 an equivalent amount of isotonic $CaCl_2$ was added to prevent any bath Ca precipitation by phosphate. Such treatment had practically no effect on the response of the uterus to pituitary extract (fig. 1).

EXPERIMENTS WITH HUMAN CEREBROSPINAL FLUID. The results obtained by the technic outlined above are summarized in tables 1 and 2. In every case the approximate absolute amount of posterior lobe extract in terms of the standard is given. Figure 1 is a tracing of a typical negative

TABLE 2

The concentration of oxytocic substance in the cerebrospinal fluid of women in labor

NUMBER	PATIENT	PREGNANCY	IN LABOR	CEREBROSPINAL FLUID		OXYTOCIC SUBSTANCE PRESENT (+) OR ABSENT (−)	MINIMUM CONCENTRATION OF FRESH P. POSTERIOR CAPABLE OF EXCITING UTERUS	ESTIMATED CONCENTRATION OF OXYTOCIC SUBSTANCE IN CEREBROSPINAL FLUID
			hours	*cc.*			$\times 10^{-4}$ *mgm. per cc.*	$\times 10^{-4}$ *mgm. per cc.*
1	NW	1	5	5.0		+	0.5	5
2	RMc	1	1.5	2.3		−	0.5	<11
3	BS	1	4	4.2		−	1.5	<18
4	RK		6	4.0		−	1.5	<18
5	MF	2	3	4.3		+	0.5	6
6	CD	3	6	4.0		+	1.5	18
7	ED		6	4.0		−	0.5	<6
8	HG	1	6	3.0		+	0.5	16
9	RW	2	6	4.0		−	0.5	<6
10	HS	1	4	4.3		−	0.5	<5
11	JB	1	4	4.4		−	1.0	<11
12	JL	4	8	4.5		−	0.5	<6
13	MY	2	6	4.5	9.1	+	2.0	14
14	EG	2	6	4.6				
15	CC		3	4.5				
16	MO	1	5	4.5	13.6	−	0.5	<2
17	LN	1	4	4.6				
18	BB	1	5	5.3				
19	RA	2	6	4.4	15.0	+	3.0	10
20	LB	3	5	5.3				

experiment. *E* and *G* represent contractions caused by 0.015 mgm. of fresh standard pars posterior. At *F* 4.4 cc. of specimen 11 (table 2) were added with practically no effect. The fluid was not washed out prior to the addition of standard extract at *G*, and there is very little change in the response of the uterus. However, an oxytocic substance is definitely present in the cerebrospinal fluid added to the bath in the experiment illustrated by figure 2. *D* and *F* represent respectively the oxytocic effects of 0.01 and 0.015 mgm. of fresh *pars posterior*, *E* that of 9.1 cc. of spinal

fluid from two different patients (nos. 13 and 14 of table 2). The total equivalent amount of *pars posterior* in the cerebrospinal fluid was calculated as being roughly 0.012 mgm. or about 14×10^{-4} mgm. per cubic centimeter.

Fig. 1

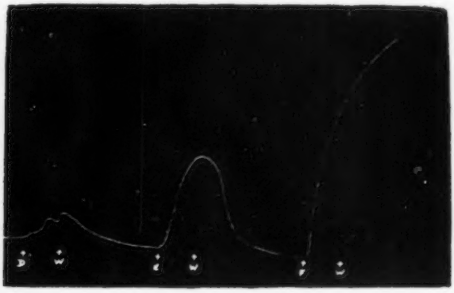

Fig. 2

Fig. 1. Oxytocic substance apparently not present in human cerebrospinal fluid. *E* and *G*, 0.015 mgm. standard fresh *pars posterior*. *F*, 4.4 cc. cerebrospinal fluid no. 11, table 2. *W*, washings with Tyrode's solution 3 minutes after *E*, and 2 minutes after *G*.

Fig. 2. Oxytocic substance present in human cerebrospinal fluid. *D*, 0.01 mgm. standard fresh *pars posterior*. *E*, 9.1 cc. cerebrospinal fluid nos. 13 and 14, table 2. *F*, 0.015 mgm. standard fresh *pars posterior*. *W*, washings with Tyrode's solution 2 minutes after addition of pituitary extract or cerebrospinal fluid.

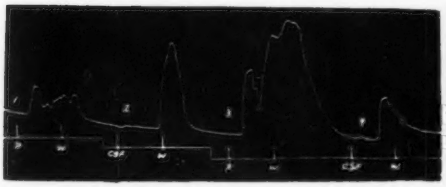

Fig. 3

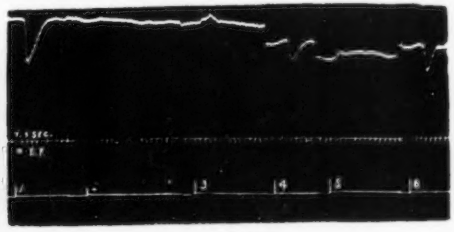

Fig. 4

Fig. 3. Oxytocic substance present in dog cerebrospinal fluid. Sensitivity of uterus increased very rapidly. *1* and *3*, 0.015 mgm. fresh *pars posterior*. *2*, 6.0 cc. dog cerebrospinal fluid, no. 7, table 3. *4*, 3.6 cc. dog cerebrospinal fluid, no. 8, table 3. *W*, washings with Tyrode's solution always done 2 minutes after addition of pituitary extract or cerebrospinal fluid to bath.

Fig. 4. Blood pressure control experiment with cat lightly narcotized by paraldehyde. Various time intervals elapsed between the intravenous injections. *1*, 0.001 mgm. histamine di HCl. *2*, 1.0 cc. dog cerebrospinal fluid, no. 8, table 3. *3*, 4.00 cc. cerebrospinal fluid of a pregnant woman. (Specimen not tested on isolated uterus.) *4*, 0.0005 mgm. histamine di HCl. *5*, 1.06 cc. cerebrospinal fluid of pregnant women. *6*, 0.0005 mgm. histamine di HCl.

From an examination of the tables 1 and 2 one can easily see that there appears to be no clear difference in the concentrations of oxytocic substance in the spinal fluids obtained from pregnant patients and from patients in labor. There is a considerable variation in the concentrations found—a fact noticed by everyone who has examined the oxytocic content of cerebrospinal fluids of animals. In a number of experiments the oxytocic principle, if present, was in too small an amount to bring about a contraction of the isolated uterus.

EXPERIMENTS ON DOG CEREBROSPINAL FLUIDS. What quantitative work has been undertaken on the concentration of oxytocic substance in the cerebrospinal fluids of animals reveals almost irreconcilable differences.

TABLE 3

The concentration of oxytocic substance in the cerebrospinal fluid of dogs

NUMBER	WEIGHT	CEREBROSPINAL FLUID	OXYTOCIC SUBSTANCE PRESENT (+) OR ABSENT (−)	MINIMUM CONCENTRATION OF FRESH P. POSTERIOR CAPABLE OF EXCITING UTERUS	ESTIMATED CONCENTRATION OF OXYTOCIC SUBSTANCE IN CEREBROSPINAL FLUID	REMARKS
	kgm.	*cc.*		$\times 10^{-4}$ *mgm.*	$\times 10^{-4}$ *mgm.*	
1	29	5.0	+	2.0	20	Unanesthetized
2	5	3.0	+	1.0	16	Unanesthetized
3	18	4.5	−	1.0	<11	Unanesthetized
4	16	4.5	−	0.5	<5	Unanesthetized Fluid *slightly* bloody
5	18	4.5	−	0.5	<5	Unanesthetized
6	20	4.5	−	0.5	<5	Unanesthetized
7	20	6.0	+	3.0	17	Anesthetized
8	17	3.6	+	1.0	16	Anesthetized
9	17	3.5	−	1.0	<14	Anesthetized Fluid *slightly* bloody

In the fluids of the cat and the dog Trendelenburg (1924) and Miura (1925) found about 3 to 4 $\times 10^{-4}$ mgm. of standard fresh *pars posterior* per cubic centimeter. Dixon (1923) used as a standard "Pituitrin" (Parke, Davis & Co.) which by the lowest estimate contains 28 mgm. of fresh *pars posterior* per cubic centimeter. He concluded that 0.5 cc. of normal dog cerebrospinal fluid *may* contain 1 to 10 mgm. of pituitrin (6 to 60 $\times 10^{-2}$ mgm. per cc.) or 150 to 1500 times as much as found by Trendelenburg and by Miura. Since the values we obtained for human cerebrospinal fluid are of the same magnitude as those of Trendelenburg and of Miura for animals we believed that it would be of value to determine again what is the oxytocic content of dog cerebrospinal fluid. *A priori* it seemed unlikely that there should be such a tremendous species difference.

The results of the experiments with absolutely fresh untreated *liquor cerebrospinalis* obtained by cisterna puncture are given in table 3. The concentrations found do not differ greatly from those encountered in human fluids. Figure 3 is an illustrative tracing of an experiment with dog cerebrospinal fluid.

CONTROL EXPERIMENTS. Jacobson (1920) found that human cerebrospinal fluids secured by ventricular or lumbar puncture seemed to contain a considerable amount of depressor substance, histamine-like in its effect on the circulation. Since histamine, as is well known, readily causes the guinea-pig uterus to contract, it was considered advisable to perform control blood pressure experiments on the cat. In these animals (2.5 to 3.5 kgm.) under light paraldehyde narcosis a definite depressor effect could always be obtained by the intravenous injection of 0.0005 mgm. of histamine—and commonly from as little as 0.00025 mgm. The experiment of figure 4 is typical. About 0.5 cc., and sometimes more, of each human and dog cerebrospinal fluid was injected intravenously into sensitive animals without a depressor action except in two instances 0.5 cc. each of fluids no. 6 (table 1) and no. 8 (table 2). In those amounts of fluid the content of histamine-like substance in terms of histamine was less than 0.00025 mgm. So an histamine-like body may have been a factor in causing the uterine contractions observed.

On the other hand a slight pressor effect was occasionally seen. The sensitivity of the uterus to histamine is known not to be parallel with that to posterior lobe extract, and unfortunately the uteri used were not tested with histamine. It seems probable, however, that histamine-like bodies usually played no part in causing the uterine contractions.

DISCUSSION. The experiments reported with human cerebrospinal fluid give no support to the suggestion of Dixon and Marshall (1924) that hypophysis secretion of oxytocic substance as measured by the latter's concentration in *liquor cerebrospinalis* plays a part in the first stage of labor. It must be remembered, however, that the fluids were collected in the first 1.5 to 6 hours of labor and that could specimens have been obtained earlier there might possibly have been an increase found.

There is good evidence that lumbar fluid contains considerably less oxytocic principle than *liquor* obtained from the cisterna whence it is commonly got in animals. Trendelenburg and Miura have found such to be the case in the dog. According to Jánossy and Horváth (1925) and Mestrezat and van Caulaert (1926), this is also true of man. Even if this is the case it seems fair to presume that with significantly increased secretion into the *liquor* at the beginning of labor a *difference* should be encountered in the lumbar fluid which in pregnancy seems to contain oxytocic substance. Attention has already been called to the fact that our experiments indicate practically a like concentration of oxytocic substance in human lumbar fluid and dog cisterna fluid.

The series of specimens from pregnant women is shorter than had been hoped. Hence it must be conceded that samples of fluid obtained considerably earlier in pregnancy might contain less oxytocic substance.

SUMMARY

1. Cerebrospinal fluids obtained from women during pregnancy and during the second to sixth hour of labor when tested on the isolated guinea pig uterus appeared to contain about the same amounts of oxytocic substance. That no increase was found speaks against the theory of Dixon and Marshall as to the interaction of the ovary and the hypophysis in terminating normal pregnancy.

2. The oxytocic substance seems not to be an histamine-like body.

3. The absolute amounts of oxytocic substance in human and dog cerebrospinal fluids are of about the same magnitude as those determined by Trendelenburg and by Miura in animals but much less than the concentrations recorded by Dixon as occurring in the dog.

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THE INFLUENCE OF HIGH SYSTEMIC BLOOD PRESSURES ON THE RIGHT VENTRICLE AND PULMONARY CIRCUIT

LOUIS N. KATZ AND CARL J. WIGGERS

From the Department of Physiology, Western Reserve University School of Medicine, Cleveland, Ohio

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Most investigators are agreed that an increase in systemic arterial pressure does not raise the pressure in the pulmonary arteries as long as the heart is not hypodynamic or its valves are not incompetent (for literature cf. Wiggers, 1921a). The observations of Führer and Starling (1913) and those of Anrep and Bulatao (1925) on the heart-lung preparation have demonstrated, however, that the pulmonary arterial pressure increases—in fact, almost proportionately to systemic pressure—even when the heart is in good condition. Führer and Starling attributed this increase to "back pressure effects", but the experimental work of Anrep and Bulatao makes it more probable that an increased coronary flow, resulting from the higher arterial pressure, is solely responsible.

It is our purpose in this report to show that while such effects *can* occur in the heart-lung preparation, they *do not* occur in the intact circulation. The dynamics of the right heart is essentially different in the two arrangements. In the heart-lung preparation the increased coronary flow is added to a relatively constant volume of returning blood, whereas in the intact circulation the increased return through the coronaries and other collateral circuits is compensated for by a reduced inflow from the inferior vena cava. Consequently, in the latter case, we may not anticipate an increase in the volume of blood entering the right ventricle when the arterial resistance is raised. A systematic analysis of the pressure changes in various parts of the systemic and pulmonary circuits, following digital compression of the aorta just above the diaphragm[1] is presented to show that these a priori conclusions are correct in fact.

Changes in pulmonary and systemic arterial pressures. The comparative pressure changes in the main pulmonary artery and aorta of dogs were recorded with optical manometers having a high "figure of merit." The technique for obtaining these curves is the same as previously described (Katz, 1925, 1927b).

[1] While our report is limited to changes in arterial resistance produced mechanically by compression of the aorta, we have observed identical effects when it is produced by reflex vasoconstriction (e. g., central vagus stimulation).

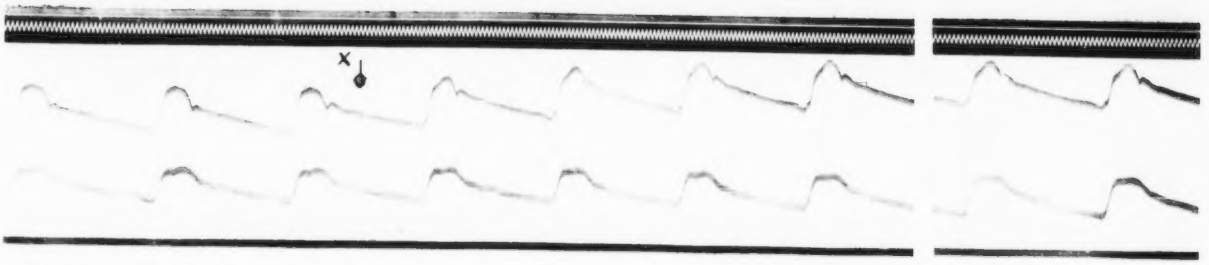

Fig. 1. Pressure curves from systemic aorta (upper) and pulmonary aorta (lower) showing immediate and stabilized effects of aortic compression Legum at *x*. Note reduction in pulmonary pressures during stabilized beats (second segment), due to slight cardiac slowing. Time, 0.02 second. Sensitivity of manometers approximately equal. $\frac{2}{3}$ actual size.

Fig. 2. Pressure curves from systemic aorta (lower) and pulmonary aorta (upper), showing effects of aortic compression (*x*), when slowing of heart develops early. Time, 0.02 second. Manometers of approximately equal sensitivity. $\frac{1}{3}$ actual size.

Figures 1 and 2 illustrate the immediate and stabilized effects found in twelve experiments. It is at once apparent, from an examination of these figures, that the alterations in the pulmonary arterial pressure are insignificant in comparison with the aortic pressure changes. In figure 1, where the heart rate at first accelerates slightly, a fleeting, barely perceptible elevation of diastolic pressure is observed in the original record. This quickly returns to its previous level. The pulse pressure gradually becomes larger and systolic pressure increases. In the stabilized beats the pulse pressure becomes still larger, but the diastolic pressure declines on account of a slight decrease in heart rate. In figure 2 the heart slows after the fourth beat. The pulmonary pressure remains practically unaltered despite the large elevation of the aortic pressures, until slowing of the heart supervenes. The prolongation of the cardiac cycle causes,

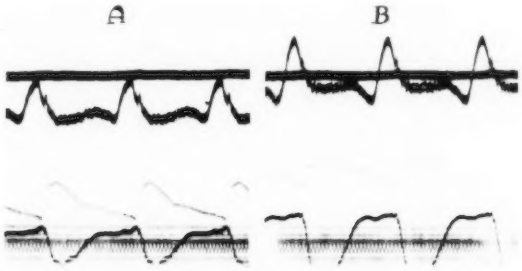

Fig. 3. Right intra-auricular pressure curve (upper), aortic pressures (middle), movements of cardiac base (lower), caudad movement is upward on curve. Segment A—control; segment B—stabilized conditions after aortic compression. Time, 0.02 second. Sensitivity of auricular manometer about 100 times that of arterial. $\frac{2}{3}$ actual size.

as expected, a fall in the diastolic and a rise in the systolic pressures of the pulmonary artery. There can be no question that in such instances the mean pulmonary pressure falls. The slowing of the heart, which accompanies an elevation of arterial pressure in the intact animal, is obviously a protective mechanism, which not only tends to neutralize the increasing systemic after-load, but actually reduces the pulmonic after-load.

Dynamics of right heart. The slight changes in pulmonary pressure obtained in the intact circulation, which are in striking contrast to the variations found in the heart-lung preparation, show that the dynamic changes in the right heart are so small as to alter the systolic output of the right ventricle very little (when the heart rate is unaltered). This is actually proven by other experiments.[2] Analysis of five experiments consistently

[2] The subsequent analysis is based on records in which no heart rate changes occurred.

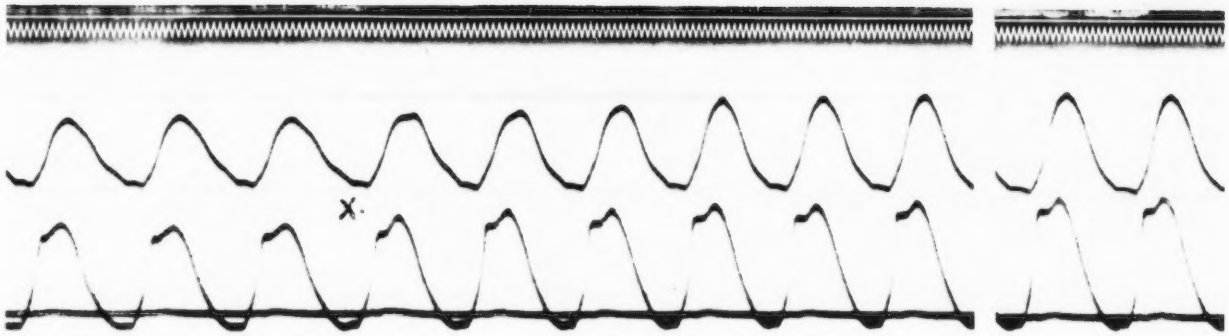

Fig. 4. Right intraventricular (upper) and left intraventricular (lower) pressure curves in relation to base lines, showing immediate and stabilized effects of aortic compression at x. Observe very slight increase in initial tension on right pressure curves, but greater elevation on left pressure curves. Time, 0.02 second. Right ventricular manometer about 2 times as sensitive as left. $\frac{1}{3}$ actual size.

showed a slight, but definite, elevation in the average pressure within the right auricle following aortic compression. This elevation became apparent within two or three beats and rose progressively to a maximum when conditions were stabilized. The two segments of figure 3 show the stabilized effects in the right auricular pressure (upper curve), which accompany the elevation of aortic pressure (middle curve). The diastolic level of the auricular pressure is not only elevated, but the amplitude of the auricular wave is increased. The mechanisms which are concerned in this elevation of pressure are *a*, an increased flow of blood through coronary and collateral circuits; *b*, the accommodation of the volume of circulating blood in a circuit with greatly reduced capacity. Both changes should augment the filling of the right ventricle to a slight extent.

On analyzing the right ventricular pressure curves in 23 animals, a slight rise in initial pressure was observed in 16 cases. In 5 animals it remained unchanged; in 2, it decreased a trifle. The typical changes are illustrated in figure 4 (upper curve). Such curves show that the pressure maximum also increases slightly, a result found in 19 out of the 23 experiments. This elevation in the amplitude of the right ventricular pressure curve is no doubt associated with a slight augmentation in the systolic discharge of the ventricle and probably accounts for the increase in pulmonary pulse pressure, noted above. Again we wish to emphasize that the magnitude of these changes is very small, and in no way comparable with those noted by Anrep and Bulatao (1925). Our findings are diametrically opposed to those reported by Straub (1917), who found that the right ventricular pressure curve *decreased* in amplitude, when the systemic arterial resistance was increased. In only two instances in our series of 23 did we obtain results at all comparable with his. We are at a loss to explain such reactions; they are not due to differences between the intact animal and the heart-lung preparation. In a series of experiments, made on 6 heart-lung preparations, we have found the increase in initial tension and amplitude in the right ventricle even greater than in the intact animal. This is shown in the four segments of figure 5 (upper curves).

Dynamics of the left heart. The pressure curves from the left ventricle show the characteristic modifications, which have been previously described by both of us, independently (Wiggers, 1921b; Katz, 1925; Wiggers, 1927; Katz, 1927a). They are also illustrated in figure 4 (lower curve) for the intact circulation, and in figure 5 (lower curve) for the heart-lung preparation. As shown in figures 4, 5 and 6, the initial pressure usually increases after the first 3 or 4 beats. The isometric pressure gradient becomes steeper and the pressure maximum is elevated. In general these observations agree with those of Straub (1917) and also with those of Patterson, Piper and Starling (1914), except that we find a more constant rise in the

initial tension and an early abbreviation of systole (cf. Wiggers, 1921b; Wiggers and Katz, 1922; Katz, 1927a).

With an elevation of initial tension in the left ventricle we expected to find an augmented pressure in the left auricle. In certain experiments this occurred, as is shown in figure 6. The left auricular pressure curve (upper tracing) rises slightly and progressively, very much as in the right

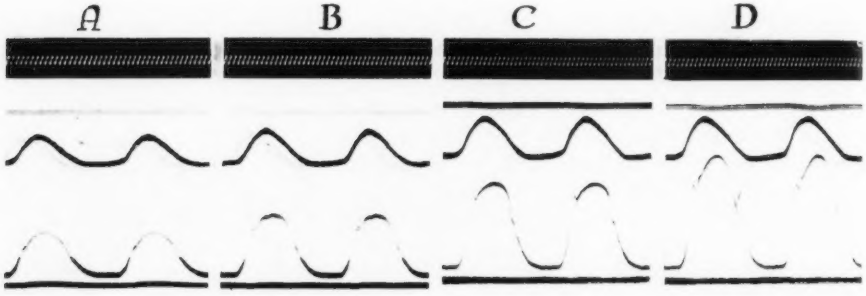

Fig. 5. Four segments of right intraventricular (upper) and left intraventricular (lower) pressure curves, showing effects of four grades of arterial resistance in a heart-lung preparation. Artificial resistance in A, 10 mm.; in B, 30 mm.; in C, 70 mm.; in D, 100 mm. Hg. Height of venous reservoir in all cases, 10 cm. blood. Sensitivity of left ventricular manometer about $\frac{1}{2}$ that of right. Time, 0.02 second. $\frac{1}{4}$ actual size.

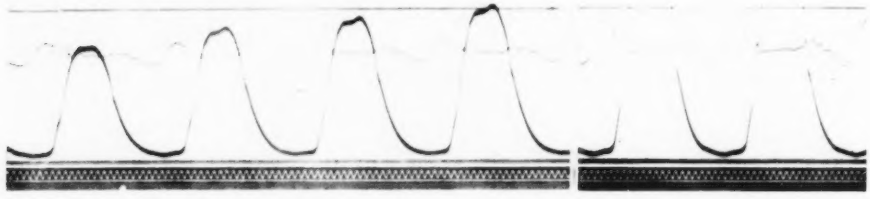

Fig. 6. Left intra-auricular (upper) and left intraventricular pressure curves (lower), showing immediate and stabilized effects of aortic compression at *x*. Sensitivity of auricular manometer about 100 times that of ventricular. Time, 0.02 second. $\frac{1}{4}$ actual size.

auricle, but to a lesser extent. Such observations are in accord with those of Straub (1917). In other experiments, however, no rise in left auricular pressure was observed, but on the contrary, the pressure fell. Such observations agree with those of Anrep and Bulatao (1925).

We are, therefore, confronted with the dilemma as to which of these contrary findings represents the actual pressure changes in the left auricle. We have given the matter careful thought and have reached the conclu-

sion that a decline of left auricular pressure is apparent rather than actual. It is due to an artefact introduced by a shifting of the ventricular base and left auricle, when the ascending portion of the aortic arch is greatly distended and probably lengthened. As the optical manometer and the cannula, inserted through one of the pulmonary veins, remain rigidly fixed, such a change in position alters the hydrostatic level and gives the false impression that pressures fall. This and other experiences have taught us that, owing to the movable position of the left auricle, quantitative pressure changes recorded from this cavity must be interpreted with considerable caution.

In support of this view may be cited the following experimental facts:

1. It is exceedingly difficult—in fact impossible—to reconcile a fall in left auricular diastolic pressure with an elevation of initial pressure in the left ventricle.

2. While the left auricular pressure curve declines rather gradually upon compression of the aorta, it rises abruptly—often within the span of a single cycle—upon decompression. Such abrupt changes cannot readily be explained on the basis of blood-flow changes, but suggest the operation of a mechanical change in the position of the auricle.

3. While the fall of left auricular pressure is common when the pericardium has been separated, it invariably increases—as far as our experience goes—when the pericardium is intact and the position of the base is much more fixed.

4. Direct tracings of the movements of the cardiac base, such as the lowest curve in figure 4, show definitely that when the pericardium is removed the base moves downward after aortic compression.

We therefore conclude with Straub (1917) that the left auricular pressures are actually raised, due to "back pressure effects." The increased "vis a fronte" thus created is not sufficient, however, to affect the pulmonary arterial pressures in the least.

SUMMARY

On the basis of pressure curves recorded from intact animals, by optical manometers having a high "figure of merit," we conclude that the following sequence of dynamic events occurs after mechanical compression of the thoracic aorta:

The small volume of residual blood retained during the first few systoles following compression causes a slight elevation of initial pressure in the left ventricle, and usually a slight increase in the diastolic pressure level within the left auricle. In the left ventricle this is accompanied by a steeper pressure gradient and a higher pressure maximum.

The increased left auricular pressure has no effect on pulmonary arterial

pressures; diastolic pressure in the pulmonary artery remains unaltered as long as the heart rate is the same, and actually falls if it slows.

The greater return of blood through the coronary and other collateral circuits often appears to overbalance somewhat the reduced flow via the inferior vena cava. As a result, right auricular diastolic and right intraventricular initial pressures are ever so slightly augmented. This leads to a slight increase in the isometric gradient of the ventricular contraction, a somewhat higher systolic summit and accounts also for a detectable elevation of pulmonary systolic pressure, often present.

Our experiments confirm the interpretation of Anrep and Bulatao that changes in pulmonary arterial pressures result from changes in the contraction of right ventricular systole, and are not due to "back pressure effects." Our results emphasize the fact, however, that in the intact circulation the changes are so slight that it requires sensitive forms of apparatus to detect their existence. The results obtained from heart-lung preparations can, therefore, not be transferred to the intact animal.

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A STUDY OF THE REFLEX TIME OF THE KNEE-JERK AND THE ACHILLES-JERK

W. W. TUTTLE, LEE EDWARD TRAVIS AND THEODORE A. HUNTER

From the Department of Physiology, and the Psychopathic Hospital, University of Iowa

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A number of investigators have measured the reflex time of both the Achilles-jerk and the knee-jerk. Up to the present time, however, the data have been collected by means of the thread galvanometer and some special recording device.

Snyder (1910), in studying the reflex time of the knee-jerk, employed a small electro-magnetic thread galvanometer. The movements of the thread were magnified by projecting its shadow, which was recorded by a special photographic apparatus. The point of stimulation was shown on the record by allowing the handle of the stimulating hammer to cast its shadow on one margin of the film just as the hammer struck the ligamentum patellae. Time was recorded by photographing the shadow of one arm of a 100 d.v. tuning fork. By means of this method twelve records were made from one subject and two from another. The average reflex time for the former subject was 0.0133 second with a range from 0.008 to 0.015 second and for the latter, 0.014 second with a range from 0.013 to 0.015 second.

Hoffman's (1910), investigation includes both the knee-jerk and the Achilles-jerk. In the case of his two subjects the reflex time of the former was found to be from 0.019 to 0.024 second and for the latter from 0.032 to 0.036 second.

Due to the apparent conflict in results and the paucity of data, the investigation herein reported was carried out. Methods were devised for recording the following:

1. The time elapsing between the delivery of a stimulus to the ligamentum patellae and the beginning of the electrical change in the muscle. This is referred to as the reduced reflex time of the knee-jerk.

2. The time elapsing between the delivery of a stimulus to the ligamentum patellae and a displacement of the leg. This is referred to as the gross reflex time of the knee-jerk.

3. The time elapsing between the delivery of a stimulus to the Achilles tendon and the beginning of the electrical change in the gastrocnemius muscle. This is referred to as the reduced reflex time of the Achilles-jerk.

The electrical change recorded in this study is referred to as the action current. The writers do not wish this term to carry any implications other than those generally accepted by physiologists.

METHOD. The action currents set up in the rectus femoris and gastrocnemius muscles were detected by a five stage amplifier. The first three stages were of the impedance coupled type comprising one stage of high and two of low amplification. These three stages which acted as the input unit were completely shielded to eliminate magnetic coupling within the amplifier itself and also stray magnetic fields and electrical disturbances. The second or output unit comprised two stages of the transformer coupled type. A volume control was placed at the second tube of the three stage unit to insure against distortion from overloading the tubes. An input transformer was used in all instances.

The recording unit consisted of three phonelescopes with an electromagnetic type of telephone receiver screwed into their backs. A beam of light was focused on the mirror of each phonelescope and reflected on the photographic film. Any rocking of the mirror caused the spot of light to move back and forth across the film.

Time was recorded by connecting a sixty cycle alternating current through a stepdown transformer (110/24) and a 2 m. f. condenser, and thence to one of the phonelescopes. The daily fluctuation charts of the alternating current showed that it never varied more than one-half of a cycle or 0.8 of 1 per cent. This amount of variation is negligible as far as our records are concerned.

Eastman standard size super speed moving picture film was used.

The electrodes leading off the rectus femoris and gastrocnemius muscles were german silver plates, twenty-seven millimeters in diameter covered with canton flannel, which was soaked in a saturated saline solution before each experiment. In case of the knee-jerk one electrode was placed in the center of the rectus femoris muscle and the other near its central end. For the Achilles-jerk, one electrode was placed in the middle of the belly of the gastrocnemius muscle and the other about four inches centrally to the first. The electrodes were, in turn, connected in series with one of the phonelescopes.

The signal circuit operated the third phonelescope. In the knee-jerk experiments the circuit was so arranged that when the stimulus was delivered the circuit was made and as soon as the foot was displaced the circuit was broken. The closing of the circuit by the delivery of the stimulus was represented by a lateral movement of the beam of light focussed on the film. The displaced light spot remained in this position until the circuit was broken by the movement of the foot, at which time it returned to its original position. Thus it is seen that when a beam of light is focused on the movable phonelescope mirror and reflected on the

moving picture film, two movements are recorded, the first for the make or stimulus, and the second for the break or response. To aid in maintaining contact immediately after the stimulus had been delivered, both the stimulating hammer and the iron stirrup carried a small amount of magnetism. This magnetism offered a little resistance to the moving of the foot so that a very slight error exists in the gross reflex time measurements.

When the stimulus was delivered, the contact of the iron hammer with the thin metal strip in closing the circuit set up a stray magnetic field which slightly disturbed the amplifier. In order to reduce this to a minimum a 200 ohm resistance was place in series and a 4 m. f. condenser in parallel with the phonelescope. The subjects were insulated from the signal circuit by a thin rubber dam placed between the thin iron plate and the ligamentum patellae in the case of the knee-jerk and between the Achilles tendon and the thin iron plate over the tendon in the case of the Achilles-jerk.

The gross reflex time of the Achilles-jerk was not measured. The signal circuit, however, in this experiment was essentially the same as for the knee-jerk except the break feature previously described was omitted.

Figure 1 shows the stability of the apparatus as assembled. Only the middle line need be considered as it represents the amplifier circuit. This picture was made with the input "short-circuited" and shows that the apparatus is absolutely free from inherent disturbances.

That the apparatus responds instantaneously is shown by figure 2. This picture was made by omitting from the signal circuit the resistance and condenser and adding increased voltage so as to produce a rather strong electrical field when the circuit was closed. The input wires of the amplifier were brought within close proximity of the place where the circuit was to be made and the circuit suddenly closed. The amplifier thus picked up the stray electrical field established by making the signal circuit and activated the amplifier phonelescope. When the signal circuit was closed its phonelescope of course was disturbed so that a comparison of the disturbances of the two phonelescopes revealed the ability of the amplifying circuit to respond instantaneously to a change in potential picked up by the input.

The subjects for the knee-jerk experiment were comfortably seated with the thigh slightly elevated to put some tension on the quadriceps muscles.

In the experiments on the Achilles-jerk, the procedure was the same except that the subject was placed in a prone position and the tension was brought on the gastrocnemius muscle by slight pressure with the hand against the sole of the foot.

DATA. Data were collected from eight normal male subjects. In the knee-jerk experiments ninety-two pictures were taken while one hundred-seven were taken of the Achilles-jerk. Figure 3 is a typical picture of the knee-jerk and figure 4 of the Achilles-jerk.

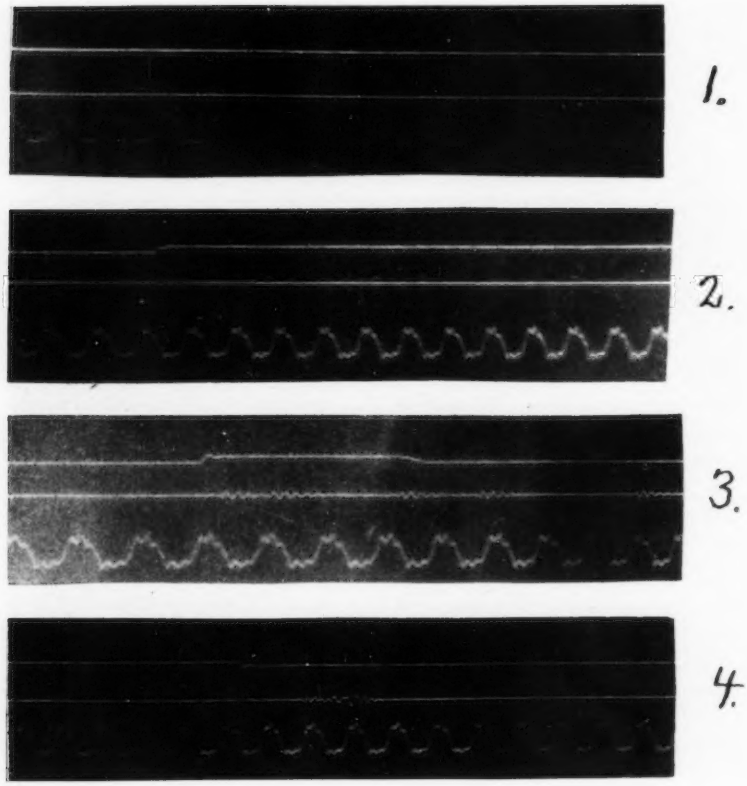

Fig. 1. The input of the amplifier short circuited to show the stability of the apparatus. The first line above the time line is controlled by the output. The second line is for the signal and is to be disregarded in this record.

Fig. 2. The instantaneousness of the response of the amplifier to a change in potential. The first line above the time line is controlled by the amplifier and the second by the signal circuit.

Fig. 3. The reduced and gross reflex time of the knee-jerk. The instant of application of the stimulus and the moment of displacement of the foot are shown on the top line. The action currents are shown on the middle line. The time is indicated in sixtieths of a second. The partials in the time line are due to the fifth harmonic of the alternating current.

Fig. 4. Reduced reflex time of the Achilles-jerk. The arrangement of lines is the same as in figure 3.

The averages and ranges for the knee-jerk response of the group are shown in table 1 and for the Achilles-jerk in table 2.

The variation in the number of pictures taken of the reflexes studied is due to the fact that a number of pictures of each group was not readable. In each case at least twenty pictures were taken.

TABLE 1

Averages and ranges of the knee-jerk responses for the entire group studied

SUBJECT	NUMBER OF RECORDS TAKEN	AVERAGE REDUCED REFLEX TIME	RANGE	GROSS REFLEX TIME	RANGE
		second	*second*	*second*	*second*
1	12	0.0189	0.0165–0.0246	0.0708	0.0583–0.0900
2	9	0.0246	0.0166–0.0333	0.1015	0.0666–0.2166
3	12	0.0186	0.0166–0.0250	0.0679	0.0600–0.0800
4	11	0.0145	0.0090–0.0183	0.0557	0.0400–0.0666
5	12	0.0202	0.0116–0.0233	0.0856	0.0618–0.1166
6	15	0.0325	0.0166–0.0400	0.1094	0.0833–0.1875
7	10	0.0105	0.0055–0.0166	0.0643	0.0500–0.0750
8	11	0.0155	0.0100–0.0183	0.0813	0.0580–0.1566
Average		0.0194		0.0796	
Range		0.0105–0.0325		0.0557–0.1094	

TABLE 2

Averages and ranges of the Achilles-jerk responses for the entire group studied

SUBJECT	NUMBER OF RECORDS TAKEN	AVERAGE REDUCED REFLEX TIME	RANGE
		second	*second*
1	12	0.0251	0.0233–0.0333
2	13	0.0336	0.0333–0.0350
3	12	0.0360	0.0333–0.0401
4	12	0.0381	0.0306–0.0466
5	9	0.0374	0.0308–0.0416
6	20	0.0375	0.0333–0.0416
7	12	0.0327	0.0266–0.0333
8	17	0.0372	0.0333–0.0400
Average		0.0347	
Range		0.0251–0.0381	

DISCUSSION. The data show that the average reduced reflex time of the knee-jerk for the entire group is 0.0194 second with a range of 0.0105 to 0.0325 second. These figures compare favorably with those of both Snyder and Hoffman. This seems to point out that the results are not conflicting but simply reveal the factor of individual differences. Some

of these differences in reflex time may be due in a small degree to differences in nerve trunk lengths. The impossibility of determining the exact length of nerve trunks involved seemed to justify the omission of an attempt to measure them. Even if one did know the exact length of the nerve trunks involved the paucity of information with reference to the synaptic, end-plate, and receptor latency in human subjects would compel one to hazard nothing more than a guess in regard to the exact time required for a true reflex.

The average gross reflex time of the knee-jerk for the entire group was 0.0796 second with a range 0.0557 to 0.1094 second. An examination of the tables will reveal that there is no correlation between the reduced reflex time and the gross reflex time in the same individual. This may be accounted for in part by the fact that uniform stimuli were not used. It seems reasonable to suppose that while the strength of the stimulus does not alter the time of arrival of the action currents it does alter the displacement time. The stronger the stimulus the greater the spread to the muscle fibrils and thus the shorter the gross reflex time.

The average reduced reflex time of the Achilles-jerk for the entire group was found to be 0.0347 second with a range 0.0251 to 0.0381 second. Not only is the average reduced reflex time of the Achilles-jerk much longer than the average reduced reflex time of the knee-jerk but every one of the eight subjects gave this relationship. Some of the difference may be accounted for by the different positions in which the subjects were placed for eliciting the two jerks. Relaxation is more easily brought about in a prone than in a sitting position. Relaxation has been shown by Jacobsen and Carlson (1925) to alter a reflex very materially. A more adequate explanation for this might be a difference in the central mechanisms involved.

The range of the reduced reflex time of both the knee-jerk and the Achilles-jerk seems to the writers to have an important bearing on the question of whether these jerks are true reflexes. If it were true that the knee-jerk and the Achilles-jerk were purely muscular responses the action currents used as an index of response would be set up by the direct stimulation of the muscle. One would then expect to find, in an experiment where a series of successive contractions are recorded, that if there were a wide range in the latent time of the responses, the shortest time would appear in the beginning followed by a progressive increase due to the phenomenon of fatigue which is a characteristic of muscular response. However the range in the responses does not follow any definite sequence but appears in an irregular order.

The relatively wide variations in range of the reduced reflex time of the responses of the same individual suggest that the knee-jerk and the Achilles-jerk not only involve a spinal reflex arc but may include a pathway

much more complex, which would furnish an adequate basis for the variations in range. Further evidence supporting this idea has been reported by Lombard (1887) who found while studying the knee-jerk that this particular reflex is altered by slight changes in environmental stimuli of almost any type.

Another finding which seems to bear on the problem of the reflex nature of the two responses is the marked difference between their reduced reflex times. This difference is too great to be accounted for on the basis of time difference in simple muscle responses or differences in length of the nerve trunks involved. In the opinion of the writers, the explanation lies in the central mechanism involved.

The nature of the action currents set up by eliciting the jerks has also an important bearing on the point of whether or not these responses are true reflexes. By referring to figure 3 it is seen that the action currents present a series of rhythmical discharges appearing as groups of the high frequency oscillations. Although the writers do not overlook the possibility of a muscle rhythm, the rates and the appearance of the group correspond to those described by Athanasiu (1925) who attributes them to Betz cell discharge. The rhythmical nature of the discharge as presented in the knee-jerk responses has been discussed in detail by the writers elsewhere (1927).

SUMMARY

1. The average reduced reflex time for the knee-jerk of eight subjects was found to be 0.0194 second with a range of 0.0105 to 0.0325 second.

2. The average gross reflex time for the knee-jerk of eight subjects was found to be 0.0796 second with a range of 0.0557 to 0.1094 second.

3. The average reduced reflex time for the Achilles-jerk of the same eight subjects was found to be 0.0347 second, with a range of 0.0251 to 0.0381 second.

4. The relatively great ranges of the reduced reflex times of the two jerks and the much longer reduced reflex time of the Achilles-jerk indicate that these phenomena are true reflexes.

5. The periodic nature of the action currents of the two responses also points to the conclusion that these jerks are true reflexes.

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DISTRIBUTION OF PANCREATIC SECRETIN IN THE GASTRO-INTESTINAL TRACT

MYRON M. WEAVER

From the Departments of Physiology of the University of Chicago and West Virginia Medical School

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As early as 1901, Wertheimer and Lepage (1), although agreeing with Popielski (2) that the pancreatic secretion induced by placing acid in the duodenum was due to a peripheral reflex mechanism, reported that the amount of secretion induced by application of acid to the intestine became steadily less, the nearer the terminal ileum was approached. The secretion was not evoked by the application of acid to the lower lengths of ileum.

The discovery by Bayliss and Starling in 1902 (3) that the injection of acid extracts (neutralized) of the duodenal mucosa would produce pancreatic secretion opened a field of controversy. That the secretion-producing substance or substances contained in the acid extract was specific for the pancreas was immediately questioned. The objections to this hypothesis were on two grounds. Secretin preparations were found to stimulate liver, stomach mucosa, salivary glands, etc. (4), (5). Furthermore, substances stimulating to the pancreas were found in many animal tissues as well as in plants (6), (5), (7).

Luckhardt, Henn and Palmer, finding that so-called "gastrin" (6) was an efficient stimulant of the pancreas and that "secretin" by the Bayliss and Starling method of preparation was a potent secretagogue for the gastric glands, concluded there was "no specificity of the so-called gastrins and secretins." They found, furthermore, that secretin may be prepared from the gastric mucosa by the Bayliss and Starling method of grinding the mucosa and boiling with acid after having destroyed the pepsin by autoclaving the tissue.

This ubiquity of pancreatic secretin in such a variety of animal tissues as well as in plants has been one of the principal arguments against its acceptance as a physiological substance, normally functioning in the body mechanism. In addition the method of preparation has been criticised as yielding a mixture of hydrolytic products in addition to whatever physiological principles the preparation might contain.

In a previous communication, Luckhardt, Barlow and Weaver (8) described a simple and rapid method of preparing a pancreatic secretin,

relatively free, not only of vaso-dilatins, but of inert substances and extraneous products of decomposition. Subsequently Weaver, Luckhardt and Koch (9) reported a method of preparing a vaso-dilatin free pancreatic secretin, this substance forming the basis for further purification.

Being in possession of a simple and relatively *physiological method of extracting and concentrating pancreatic secretin*, it seemed important to investigate once more its distribution in various animal tissues and especially in the mucosa of the gastro-intestinal tract. In investigating this question Lalou (10) made extracts from the mucosa of various parts of the gastro-intestinal tract by the method of grinding the mucosa and boiling with dilute acid. His observation was that duodenal extracts were ten times as active as extracts of ileum. Similarly Mellanby (11) using alcoholic extracts has reported that secretin may be obtained from the upper two-thirds of the small intestine of the goat, from the upper ten feet of the small intestine of the pig, and from the duodenum only in the cat.

METHODS. *1. Preparing dogs for injection.* Dogs of nearly uniform size were used, i.e., about 15 kilos in weight. They were lightly anesthetized with ether and as soon as unconscious were given barbital-sodium intravenously. The dose used was 200 to 250 mgm. per kilo body weight. The ether was removed immediately upon beginning the barbital injection.

This very rapid method of inducing surgical anesthesia has many advantages. Ether is used so briefly that it can scarcely be said to furnish a disturbing factor in an experiment extending over a period of several hours. But by using ether at the start it is possible to bridge over the stage of cerebral excitation which is a frequent accompaniment of barbital administration when the drug is given by stomach tube or even intravenously. It has been a common experience in the past to find a dog which has passed through this excitement stage refractory and sometimes useless insofar as possibility of eliciting a pancreatic secretion is concerned. The nature of this inhibition is unknown to the author.

2. Extraction of tissues. The method of simple exposure to 0.4 per cent hydrochloric acid was used throughout.

In the case of the stomach, intestines and large blood vessels, the procedure was as has been previously described (8). After washing the lumen of the viscus with cold tap water, one end is closed with a hemostat, the acid introduced by means of a pipette and the other end closed with another hemostat. Surfaces such as the buccal cavity, peritoneal cavity, pleural cavity, etc., were gently sponged with cotton wet with physiological saline and the acid applied by allowing it to rest in natural cavities. In the case of the buccal cavity this involved tying the mouth open and placing a cork in the pharynx. Extraction of the liver, cardiac muscle, etc., was accomplished by grinding the organ in a meat chopper and extracting in the cold with 0.4 per cent acid.

Particular interest attached to a comparison of the activities of preparations made from the lining tissues of the various portions of the gastro-intestinal tract. Extracts were made of buccal mucosa, esophagus, fundus of stomach, pyloric antrum, the small intestine by fifths, i.e., duodenum and intestine[2], intestine[3], intestine[4], intestine[5], and colon-rectum. In dividing up the small intestine it was deemed advisable to divide it into five equal lengths. In order to obviate the criticism that different amounts

TABLE 1

Gastro-intestinal extracts

VISCUS	NUMBER OF EXPERIMENTS	EFFECT ON BLOOD PRESSURE OF ACID EXTRACT OF VISCUS	SECRETAGOGUE ACTIVITY OF ACID EXTRACT OF VISCUS	PRECIPITATE WITH NaCl	SECRETAGOGUE ACTIVITY OF PRECIPITATE
Buccal cavity.....	6	None	None	None	
Esophagus..	10	None	None	Slight	None
Stomach (entire)...	6	0—Slight fall	0—Slightly active	Slight	*
(a) Cardia..	4	0—Slight fall	None	Slight	None
(b) Pylorus.	4	0—Slight fall	0—Slightly active	Slight	*
Duodenum..	14	Fall in blood pressure 40–60 mm. Hg. average	Very active	Precipitate average 0.20–0.30 gram per 25 cc. acid extract	85.5 drops
Intestine[2]...	14		Very active		45.2 drops
Intestine[3]...	14		Active		23.0 drops
Intestine[4]...	14		Slightly active		15.1 drops
Intestine[5]...	14		0—Slightly active		None†
Colon-rectum....	6	Fall, 60–100 mm. Hg.	None	Heavy precipitate	None

* Upon two occasions there followed a definite secretion of the pancreas upon injection of acid washings of the stomach mucosa. One of these preparations was an extract of the entire stomach mucosa and the other two were extracts of the pyloric antrum mucosa.

† Upon a few occasions there was observed a slight pancreatic response following injection of preparations made from the last fifth of the small intestine. The usual finding was that preparations from that region were inactive.

of mucosa were being extracted by equal quantities of acid, the mucosa was scraped from the lengths of intestine of a number of dogs and weighed and a rough ratio established between the amounts of mucosa extracted. The ratio established was 4:3:3:3:3, the lower lengths of gut receiving the benefit of the slightly larger amount of acid per gram of mucosa extracted. Varying amounts of acid were used in extracting esophagus, stomach and colon-rectum in an effort to detect any activity at all.

3. Preparation of extracts for injection. In the further preparation of extracts for injection, the following factors were considered:

1. In using secretin preparations of low potency, the pancreas may fail to be stimulated by moderate doses, responding, however, to the injection of larger quantities of the preparation. Thus the presence of some activity in preparations of low-grade efficiency might be overlooked unless provision be made for so concentrating the active principle that its presence be made apparent upon injection.

2. At the same time, with powerful preparations it is easy to obtain a secretion-rate which is maximal for the gland, the increase of quantity of secretin injected producing no increase in rate of secretion. Since all preparations injected should for the sake of comparison be concentrated to the same degree, this latter factor made it necessary to avoid concentrating the preparation too much.

From experience it was found advisable to use the precipitate from 25 cc. of the secretin solution, obtaining this precipitate by saturating the secretin with sodium chloride and taking up the filtered precipitate in 5 cc. of physiological saline warmed to body temperature. While such a solution was rarely free of a trace of vaso-dilatin, it nevertheless possessed the advantage of being in neutral solution and there was a minimum loss of activity such as follows washing and reprecipitation. This was of prime importance in making a comparative study.

The method outlined above could be used only in comparing secretin solutions which yield a precipitate and was therefore applicable to studying the gastro-intestinal tract from the stomach to the rectum. It is noticeable however that only preparations which yielded a precipitate with NaCl showed secretagogue activity. At the same time there are instances where a heavy precipitate is thrown down which proves to be inactive (see Results).

4. Direct injection of the pancreas. In the past it has seemed desirable to effect a more direct injection of the pancreas than that which is commonly accomplished by injection of substances into the systemic circulation. Among other reasons it has been shown by Cowgill and Deuel (12) that when secretin solutions are injected into the portal vein, the pancreas fails to respond until the liver has been saturated. However, as Farrell and Ivy (13) have pointed out, this might be due to adsorption of the secretin to impurities in the secretin solutions injected. As a matter of fact it seems almost certain that the active secretin principle may be adsorbed to the material which is in suspension in the original secretin solution, especially if this has been prepared after the method of Bayliss and Starling.

Direct injection of the pancreas was made especially desirable in this work inasmuch as it was conceivable that a given preparation, say of

stomach mucosa, while proving inactive upon introduction (with attendant dilution) into the general circulation, might upon direct injection of the pancreas show some activity. In view of the extreme sensitivity of the pancreas to any interference with its blood supply, it was found practical to proceed as follows:

The celiac artery is exposed and the splenic artery cannulated for injection, close to its exit from the celiac. The left gastric is tied off and a lifting ligature placed under the celiac. By temporarily occluding the celiac and injecting into the splenic, preparations may be introduced into the hepatic artery. Escape of the solution through the right gastric, gastro-epiploic or cystic arteries is prevented by closing these arteries with hemostats. The secretin is thus introduced directly into the pancreatico-duodenal artery which is the principal arterial supply of the pancreas in the dog. Suitable precautions are necessary to prevent coagulation in the splenic artery and this may be accomplished either by hirudinizing the animal or, what has proved more satisfactory, connection of the cannula in the splenic with an apparatus similar to that used in recording blood pressure so that the blood from the splenic is allowed to come in contact with a suitable anticoagulant in the cannula. Injections may be made by closing off the lead to the blood pressure apparatus and injecting through the side arm of the cannula. The arterial supply to the pancreas is thus only temporarily interrupted and the circulation may be maintained for a period of hours. Recently Babkin and Starling (14) have used a similar method for perfusing the pancreas.

Results. From the accompanying table it is evident that buccal acid extracts are inactive when injected in the original acid solution and furthermore yield no sodium chlorid precipitate so they cannot be concentrated by this method. The above applies also to extracts of the esophagus. The acid washings of the stomach may show activity, this activity coming from the region of the pyloric antrum.

The principal location of secretin in the gastro-intestinal tract is in the duodenum and the small intestine immediately ensuing. The figures in the tabulation are the average of 14 experiments, these experiments having been selected from a larger series. The experiments were chosen because *a*, they represent a complete set of figures embracing all portions of the gastro-intestinal tract, and *b*, there were control injections before and after running the series to show that the pancreas was responding in a constant manner. In order to effect a further control it was the practice to vary the order of injections, i.e., duodenum, intestine^{2},—intestine^{5}, colon. Then colon, intestine^{5}—intestine^{2}, duodenum and lastly duodenum, intestine^{3}, colon, intestine^{2}, intestine^{4}, intestine^{5}.

As examples of the maximum and minimum responses which are averaged in these results the following may be cited:

	MAXIMUM	MINIMUM
	drops	*drops*
Duodenal extract	207	31
Intestine[2] extract	87	9
Intestine[3] extract	30	7
Intestine[4] extract	18	1
Intestine[5] extract	0	0
Colon-rectum	0	0

As noted above, these figures represent the number of drops of pancreatic secretion induced by injecting the precipitate obtained from 25 cc. of the acid washings of the viscus named, the precipitate being redissolved in 5 cc. of physiological saline for injection.

Under *Methods* was described a means of direct injection of the pancreas, this being used in an effort to determine activity by direct introduction of the substances investigated into the pancreas. While by this method it was possible under favorable conditions to treble the response to the pancreas to the injection of a given quantity of secretin, it was never possible to show any activity in preparations which had been found inactive by the other method of injection.

An additional check upon this comparative study was effected by tying the intestine into five roughly equal lengths and applying 0.4 per cent HCl to the lumen of the compartments thus formed. The results were as follows:

	drops
Duodenal application	27
Intestine[2] application	16
Intestine[3] application	6
Intestine[4] application	2
Intestine[5] application	0

These results are in accord with those of Lalou (10), Mellanby (11), and Wertheimer and Lepage (1), previously reported.

Other extracts prepared were of pleura, peritoneum and endothelium of large blood vessels (aorta and vena cava) and the lining of the trachea and bronchi. Liver, cardiac muscle, skeletal muscle, omentum, salivary glands, lymph glands were extracted but the preparations showed no activity.

CONCLUSIONS

1. Pancreatic secretin as extracted by simple washing of the tissue with 0.4 per cent HCl is found only in the gastro-intestinal tract, occurring principally in the duodenum and then in rapidly decreasing quantities down the small intestine, being absent or nearly so from the last fifth of the small

intestine and entirely absent from the colon-rectum. These statements apply to the dog which was the animal used for the investigation.

There may be found small quantities of this substance (pancreatic secretin) in the stomach mucosa, occurring principally in the mucosa of the pyloric antrum.

2. The pancreas has been shown to respond to direct injection of vasodilatin-free secretin.

It is a pleasure to acknowledge indebtedness to Doctor Luckhardt and to Doctor Carlson for their suggestions and helpful criticism.

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A ROENTGENOLOGICAL STUDY OF GASTRIC HUNGER MOTILITY IN A SERIES OF HEALTHY MEN

F. T. ROGERS AND C. L. MARTIN

From the Laboratories of Physiology and Roentgenology, Baylor University

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The specific purpose of the work herewith presented is to describe that type of gastric motility designated by Cannon, by Carlson and by their associates as hunger contractions. The method employed by these investigators consisted in introducing a rubber balloon into the stomach of the fasting individual which is then distended with air. Graphic registration of changes in gastric pressure caused by the gastric contractions are then recorded. This indirect method, delicate as it is and much as it has revealed, does not tell us whether the contractions are localized in any definite regions of the stomach, either antrum or fundus, or whether they are contractions of large sections of the stomach as a whole; whether they are peristalses, tonus changes, or complex mixtures of such activities.

On the basis of his studies by the graphic registration method, Carlson (1) recognized the following types of intra-gastric pressure changes associated with hunger: a pulse rhythm, a respiratory rhythm, the "twenty seconds' rhythm" and the "thirty seconds' rhythm." He recognized that the twenty seconds' rhythm might be pressure changes due either to peristalsis of the antrum or to tonus changes of the fundus. Carlson, Orr and McGrath (2) found that in dogs this rhythm is exhibited by the isolated Pavlov pouch and therefore concluded that it represented true tonus changes of the fundus. The thirty seconds' rhythm or the hunger contractions proper were classified as types I, II and III, according to the intensity of the contraction and the degree of gastric tonus as judged by the maintained intra-gastric pressure. Type I contractions are relatively feeble. Type II contractions are more powerful and occur in groups with increasing frequency which terminate in a period of incomplete tetany. Type III contractions are rapidly repeated contractions associated with increased gastric tonus.

The graphic method alone can not yield information as to the exact nature of the hunger contractions. On the basis of his experimental studies, Carlson speaks of them as "fundus contractions." Owing to the fact that the introduction into the stomach of any solid or liquid materials inhibits the hunger contractions, this type of gastric motility can not be

studied by the customary x-ray methods of examination after giving a bismuth or a barium sulphate meal. In 1916 Rogers and Hardt (3) reported the results of a single x-ray observation of hunger contractions as they were rendered visible by coating a rubber balloon with a paste of bismuth sub-nitrate. The present writers have doubted the real value of this single observation because the mass of such a bismuth paste balloon is such that one may justifiably question whether or not the resulting contractions are other than the normal gastric peristalses induced by semi-solid material in the stomach. Rogers and Hardt also observed that in the fasting young dog, examined by the bismuth balloon method, hunger contractions consisted of spasmodic contractions of practically the entire stomach. Against both these observations criticisms may be advanced as to their general applicability to man. Hence we have sought a method of studying this hunger motility by the x-ray without the use of the heavy opaque substance as customarily employed in gastro-intestinal work.

During recent years there have been improvements in roentgenological technique in clinical work which have rendered the study of gas shadows entirely feasible. It is now possible to observe directly the outline of the air-distended rubber balloon in the stomach by fluoroscopy, or to study the motility by making a series of roentgenograms while the hunger contractions are being simultaneously recorded by the graphic method. Recently Rogers and Martin (4), (5) have described the hunger motility as observed by this method in a single subject. Since the observations on a single individual may not be applicable to others, we have extended the observations to a group of five healthy men. Two of these men (M. H., age 28, and L. A. B., age 23) are medical students; two of them (R. W. L., age 27, and F. T. R. age 37) are college instructors; and one (F. H., age 37) is a research student.

Methods. The radiographic work was done with double-coated films loaded between intensifying screens in aluminium cassettes. Half-second exposures were used with 40 milliamperes flowing through a fine focus universal Coolidge tube operated at a voltage just high enough to break down an air gap of 8 inches between blunt points. A permanent filter of aluminium having a thickness of 0.5 mm. was placed beneath the tube to increase the safe number of exposures. The patient lay comfortably in a supine position on a Potter-Bucky diaphragm and a target cassette distance of 25 inches was used.

The outlines of the air-filled stomach were found to be very indistinct when viewed on the fluoroscopic screen. However the placing of a stationary Potter-Bucky grid with very narrow grid spaces, between the subject's body and the screen increased the contrast to such an extent that satisfactory observations could be made. The grid was supported during each experiment so that it rested just above but not in contact

with the abdomen of the supine subject on the horizontal fluoroscopic table. Very faint waves and antral contractions falling directly over the spine were not distinct but the deeper waves could be seen with ease except when a large amount of intestinal gas was present.

The greatest care was exercised in keeping the x-ray exposures within the safe limit for the skin of the subject. During the fluoroscopic work the actual time of exposure was recorded with a stop watch and the work was halted when the skin of each subject had received a calculated dose of x-rays equal to two-thirds of a skin erythema dose. In order to avoid the cumulative effects of x-rays, no subject was reëxamined until at least a month had elapsed since the previous examination.

Aside from the precautions essential to the x-ray technique, it is necessary that the subject of the observations be able to swallow and retain the rubber balloon with no discomfort, and furthermore that he feel no anxiety in being exposed to the x-ray. In one individual in this series, this last factor was such as to seriously depress the hunger motility. Having swallowed a rubber balloon (condom) connected by soft rubber tubing with a chloroform manometer so as to make a graphic record of the gastric hunger motility, the subject then lay down on the x-ray table, and the fluoroscopic or radiographic examination was made simultaneously with the graphic registration of the hunger contractions. The volumes of the distended balloons at the pressures employed varied from 100 to 150 cc. The total number of observation periods was fourteen, divided as follows: five periods on F. T. R., four periods on L. A. B., three periods on M. H., and one each on R. W. L. and F. H. In each instance the subject ate no food for twenty-four hours preceding the x-ray examination.

For clearness of presentation, the figures in this report are tracings of the outlines of the stomach copied from the roentgenograms. As anatomical landmarks, the location of the 11th and 12th ribs and vertebrae are indicated in each figure. In all cases the fundic end of the stomach is to the left, and the pyloric end of the stomach is to the right side of the drawings. The numbers in each figure indicate the time instant on the corresponding tracing at which the roentgenogram was made.

Results. *Position of the fasting stomach.* In four of the individuals examined in the reclining position, the stomach with the air-distended balloon was found to lie nearly transversely across the spinal column at the level of the 11 and 12th thoracic vertebrae (fig. 1). In the case of one man (F. H.) also examined in the reclining position, the balloon was found to lie in a nearly vertical plane parallel to the spinal column (fig. 2).

Twenty seconds' rhythm. We have frequently examined the stomach both fluoroscopically and by radiographs, while the twenty seconds' pressure rhythm was being recorded. The only observed motility of the stomach at this time is that of shallow peristaltic waves running over the

lower two-thirds of the stomach (see *7* and *8* in fig. 7). If this peristalsis is occurring at such a rate that a second or third wave begins before the first has disappeared it causes a further prolonged increase in the intra-

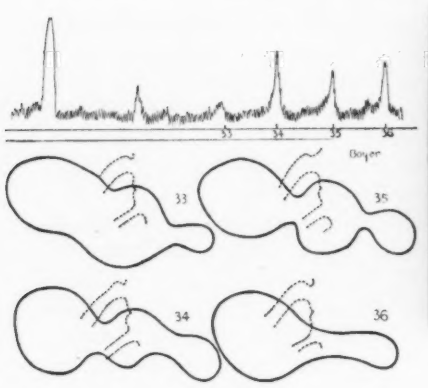

Fig 1

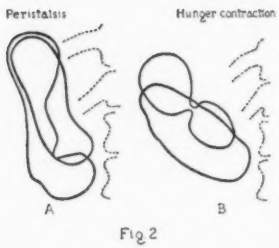

Fig 2

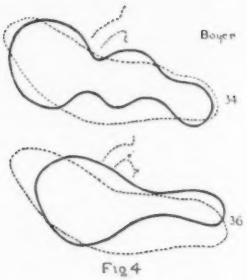

Fig 4

Fig. 1. The onset of a period of hunger contractions. Roentgenograms of the stomach were made at the times indicated by numbers on the graphic tracing. The outlines of the stomach at these times are shown in the lower part of the figure. The position of the 11th and 12th ribs is indicated by dotted lines in all figures.

Fig. 2. To show the vertical position of the fasting stomach in F. H. On the left side is indicated the outlines of the stomach as it exhibited peristalsis, recorded on the graphic tracing as "twenty seconds' rhythm." On the right side are shown superimposed tracings of the outline of the stomach, first in a period of rest and secondly during a strong hunger contraction.

Fig. 3. A period of strong hunger contractions in L. A. B. terminating in a period of incomplete tetany. Roentgenograms of the stomach were made at the times indicated by numbers. The outlines of the stomach at these times are shown in figures 5 and 8.

Fig. 4. Outlines of the stomach made during type I hunger contractions, superimposed on the outlines of the stomach in the intervals of rest between hunger contractions. The peristalsis and the tonic constriction of the lower half of the stomach are evident. Note also the distention and the apparent shortening of the fundic end of the stomach.

gastric pressure. It has been observed that repeated peristaltic waves occurring at irregular intervals may in this way cause short periods of increased intra-gastric pressure simulating a "tonus rhythm." However, there is another element in the tonus rhythm that appears with the onset of hunger contractions.

Type I hunger contractions. The onset of hunger pains judging from the graphic record is marked by waves of increasing intra-gastric pressure or tonus rhythm, which culminate in stronger contractions designated by Carlson as type I hunger contractions. As observed fluoroscopically, the beginning of a type I contraction is marked by a constriction of varying intensity and width, in the lower third of the stomach. This is accompanied by the pressing of the air backward with a distention of the fundic portion of the stomach. This is followed by a peristaltic wave which starts in the upper half of the stomach and sweeps toward the pylorus. In the meantime there may be maintained tonic or circular contraction of the lower half or third of the stomach (see nos. *34* and *36* of fig. 1, fig. 3, fig. 4 and fig. 5); or this antral contraction may relax in front of the advancing peristaltic wave (fig. 2 and nos. *11* and *9* of fig. 7).

The above description applies to most of the weaker hunger contractions observed but at times we have seen other types of activity. These consisted of circular contractions of the lower third of the stomach without the appearance of fundic peristalses. These antral contractions were felt by the subject of the experiment and were thought to be hunger contractions.

Still another type of contraction that may be of considerable significance has been seen at times. This consists of an apparent general shortening of the entire stomach. (See no. *36* in fig. 4 and no. *7* in fig. 5.) There may however be an element of doubt concerning this apparent shortening for it may be due to a change in the curvature of the long axis of the stomach. If it be a real contraction phenomenon it is an important element in the "tonus" changes associated with hunger contractions.

Type II contractions. These are of greater amplitude and frequency than type I contractions. The same elements are present as in type I contractions but they are of greater intensity. These elements are twofold, first a contraction of the antral portion of the stomach frequently followed by distention of the fundic portion of the stomach and then by a deep peristaltic wave which arising high in the fundus completely cuts across the lumen of the stomach (figs. 6 and 7). In some cases we have observed these waves starting at or near the cardiac sphincter, run over the entire stomach, terminating in a constriction that obliterates the lumen of the lower third or half of the stomach (see no. *12* in fig. 7).

Periods of tetany. In three instances we have had the opportunity to study the periods of incomplete tetany that terminate the periods of type

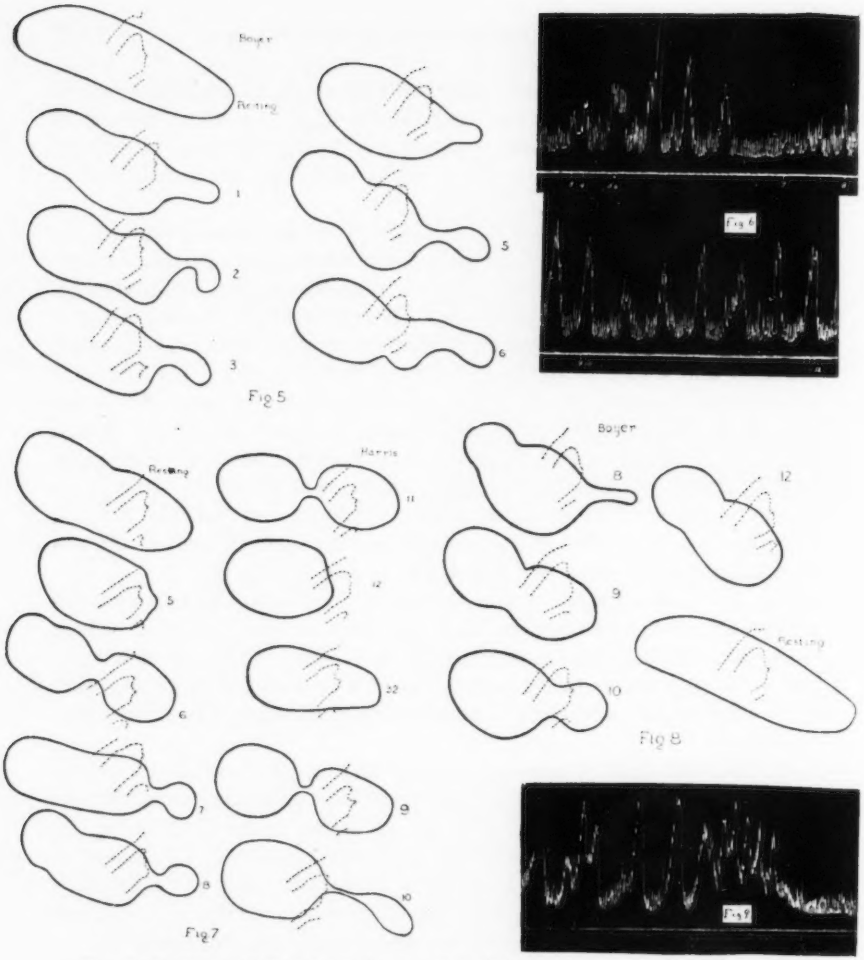

Fig. 5. Outlines of the stomach at rest and during hunger contractions at the times indicated by numbers in the graphic tracing of figure 3. The predominating activity of the lower half of the stomach is clearly indicated in this figure.

Fig. 6. Tracing of hunger contractions of M. H. Radiographs of the outline of the stomach at the times indicated by numbers on the tracing are shown in figure 7.

Fig. 7. The outlines of the stomach at rest and at varying times during the play of hunger contractions recorded in figure 6.

Fig. 8. The outlines of the stomach in the period of incomplete tetany that terminated the group of type II contractions shown in figure 3. It will be observed that this period of tetany is marked by a spasmodic contraction of the lower third of the stomach, plus peristaltic waves arising near the extreme upper end of the stomach.

Fig. 9. Incomplete tetany terminating a period of hunger contractions in F. T. R. This period of motility, marked *x*--------*x*, was observed fluoroscopically. It consisted of a series of rapidly occurring, deep peristaltic waves, arising high in the fundus and terminating in strong contractions, which completely cut off the lumen of the antrum.

II contractions. In one of these cases studied fluoroscopically and in one case studied by radiographs it was seen that the tetany consisted of a series of deep peristaltic waves cutting completely across the lumen of the stomach, arising high in the fundus and occurring at such a rate that a new contraction wave began before the relaxation of the preceding one was complete. In the third case studied by radiographs, we found a maintained contraction or spasm of the lower third of the stomach with a simultaneous play of peristaltic waves in the fundic end of the stomach (see figs. 3 and 8). In this instance it is clear that the increased intragastric pressure or "tonus" on which the individual contractions are superimposed in the graphic tracing, is due to increased tonicity of the lower end of the stomach.

This study has not been extended to children or to animals. Possibly in such cases the fundic end of the stomach may exhibit a higher degree of activity than has been observed in the adult men studied in this work.

SUMMARY

Radiographic and fluoroscopic examinations of gastric hunger contractions have been made on five healthy men with simultaneous graphic registration by the rubber balloon and manometer method.

This gastric hunger motility is a mixture of at least two types of activity; first, hyper-peristalsis and second, tonic or circular contraction of the lower third or antral end of the stomach. Visible tonic or maintained contractions of the fundus were not found to be constant characteristics of hunger contractions; sometimes this portion of the stomach has been found dilated or distended by the greater degree of contractility of the lower portion of the stomach; at other times there has appeared an apparent shortening or contraction of the entire fundus.

The twenty seconds' pressure rhythm of the graphic tracing is associated with simple peristalsis. In type I contractions there occurs first a shallow peristaltic wave followed by a strong antral contraction which is immediately followed by a deep peristaltic wave. The type II contraction is similar to that of type I. There occurs first a contraction of the antrum but the following peristaltic wave may originate high in the fundus, even at or near the cardiac sphincter, and sweep over the entire stomach terminating in an antral contraction completely obliterating the lumen of the lower half or third of the stomach. Periods of gastric tetany, as studied by the graphic method, are characterized by repeated contractions with maintained "tonus" or increased intragastric pressure. This maintained intra-gastric pressure, it can be seen fluoroscopically or from radiographs, is due to either or both of two factors; first, increased constriction of the antral end of the stomach and second, hyper-peristalsis at such a rate that a second deep wave appears before the preceding one has disappeared.

The most striking feature of this work has been the observation of the complete obliteration of the lumen of the lower portion of the stomach at the height of the hunger contraction. It is at this time that the subject feels the contraction most intensely.

We wish to acknowledge the valued voluntary assistance of the following men whose coöperation made this study possible: R. W. Lackey, L. A. Boyer, Marshal Harris and Frederick Hoelzel. We also wish to acknowledge the financial assistance of a grant from the American Medical Association for the prosecution of this work.

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STUDIES IN EXHAUSTION DUE TO LACK OF SLEEP

I. Introduction and Methods

THEODORE H. BAST and A. S. LOEVENHART

From the Departments of Anatomy and Pharmacology of the University of Wisconsin Medical School

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Functional disorders of the central nervous system, particularly of the type noted in impending nervous exhaustion with accompanying insomnia, are of the utmost importance in medicine. The lack of accurate criteria to predict the onset of functional nervous disorders as well as other functional diseases has impeded the development of a rational prophylaxis or of a satisfactory treatment. Experimental studies have not been sufficiently comprehensive to furnish a detailed picture of the morphological changes, if any, which accompany the functional disturbances; nor has the symptom complex of nervous exhaustion, when carried to collapse in experimental animals, been described. Such studies may be expected to furnish an insight to the conditions existing in "nervous breakdown" as observed clinically. There is also great need for an accurate evaluation of the rôle of nervous exhaustion in acute and chronic diseases.

In all cases of disease in the human there is probably a fatigue or exhaustion factor which modifies the symptomatology, resistance to infection, and functional activity of different organs. An evaluation of the fatigue factor, both from the standpoint of diagnosis and of treatment, would be of the greatest importance and may be furnished by properly devised experiments. One should begin with the study of the simplest type of fatigue. An experimental animal should be maintained without sleep, the end point being death, in order to obtain the most exaggerated picture possible. During the production of such fatigue, the symptoms shown by the animal should be carefully noted, and along with these functional studies, morphological examinations should be made post-mortem, in which meticulous care must be taken to prevent post-mortem changes or the production of artifacts. A correlation may then be possible between symptoms and structural changes, if any such relation really exists. Finally, control experiments must be performed keeping the animals under identical conditions without loss of sleep to show the adequacy of the histological methods used in preventing post-mortem change and the

formation of artifacts. These are the fundamental requirements of experimental procedure in this difficult field, if there is to be any general acceptance of the results of the work. *A priori*, we would not expect all animals to die with the same symptomatology and pathological findings because of individual variation in the animals. Thus, exhaustion due to loss of sleep in certain individuals would probably be due to decreased resistance to infection so that they might die of different infections. In others, functional, circulatory, digestive and nervous disturbances might be the important factor in causing death.

Attempts to study fatigue are not new. Ever since the formulation of the Neuron Theory in 1891, the observations of Nissl (1896) on nerve cell structure, and the effect of injury on nerve cell protoplasm, fatigue experiments of various types have been attempted. The outstanding work on the physiological effects of loss of sleep has been done by Kleitman and his associates (1923).

Work on the morphological effects of fatigue on nerve cells has been carried on by a number of workers, foremost among whom are Hodge (1894), Luxemberg (1899), Vas (1892), Dolley (1911), Crile (1921), Lambert (1893), Kocher (1916), Legendre (1908) and Pieron (1908). Hodge studied Nissl bodies in the nerve cells of sparrows in the evening after a day on wing, and also in the morning after a night's rest, and he reports protoplasmic changes. Dolley, Crile, Vas and others produced fatigue by excessive exercise over a period of three or four hours. They claim to have been able to show an increase in the size of the cells, a disturbance in the relation between the nucleus and cytoplasm and a hyperchromatism followed by a hypochromatism. With apparently the same type of material and technique, Kocher and Lambert, working independently, were unable to find any constant deviation from that of their corresponding normal control cells. Somewhat different results were obtained by Hodge, Luxemberg, Legendre, Pieron and Nissl who observed a change in the cell which consisted of a shrinkage of both cytoplasm and nucleus. The work of these three groups is typical of the methods and results of studies in this field. Besides studying the nervous tissue, Crile also studied some of the other organs in fatigued animals and reports marked changes in the adrenals and liver.

Methods. It was decided to devote our initial efforts to the study of symptoms developing in animals exhausted through lack of sleep and to investigate the morphological changes in their tissues, especially in the central nervous system, adrenals, and thyroid. Later it was contemplated to extend the work to include various biochemical, physiological and pharmacological observations, together with a study of any change in the power of the animals to resist infection.

Rabbits were used throughout. The prevention of sleep was accom-

plished by placing the animals in cylindrical cages bearing a horizontal axis around which the cages revolved. A pair of these cages is shown in figure 1. They are eighteen inches in diameter and eighteen inches long. Two are mounted on a wooden frame together with a $\frac{1}{6}$ horse power motor and a reducing worm gear. The ends of the cages are made of three-quarter inch boards and the sides are of wire screen of one-quarter inch mesh. The cages are mounted on a one-half inch steel rod which passes through the center of the cylinder. This rod is provided with a pulley which is connected to the reducing gear by means of a leather belt. The second cage is rotated from the first cage, being connected with it by a leather belt and pulley wheel.

During the experimental runs, the cages were rotated at a speed of 1.1 revolutions per minute. This forced the rabbits to change position about eight times per minute. The movement usually consisted of the animal taking a step forward in a leisurely manner. The total distance covered in twenty-four hours was 7,500 feet or 1.42 miles. By using this slow speed of revolution, the factor of physical exertion and muscular fatigue was reduced to a minimum.

Underneath each cage there is a specially built funnel covered with a twenty mesh copper screen which permits the collection of urine and feces separately. Within each cage and suspended from the center rod is a two compartment feed box which is constantly kept supplied with food and water for the animal. The food supply box slips around on the center rod as it rotates. One end of each cage is provided with a six inch square sliding door.

In the earlier experiments, it was found that the animals developed sores on their feet as a result of sliding on the wire screen when they became weary. In order to avoid this complication, soft leather boots were placed on all four feet of each animal.

The selection of the rabbits was made with much care. They were all free from ear mites and from obvious infections or abnormalities. They were subjected to a careful physical examination, including a red blood cell count, hemoglobin determination, and a total and differential white cell count. They were usually of the same sex and of like size and character. Seven comparable rabbits served as controls, living in similar cages on the same ration and in the same room during the entire period of the experiment, but were not rotated.

There was a four or five day observation period on each animal before the rotation began. During this time clinical observations were made twice a day, the pulse rate, respiratory rate, and body temperature being taken. The urine was examined for albumen and casts daily during this period. Any abnormal animals were discarded.

During the period of rotation, the animals were examined several times a day at the beginning of the experiment. The rotation was stopped thrice daily to replenish food and water and to remove the rabbit for observation. As the experiment advanced, the animals were observed more frequently and when they began to show marked symptoms of exhaustion, they were watched almost continuously until they collapsed and were removed.

General comment. The behavior of the animals in the rotating cages is interesting. They always face in a direction opposite to the movement of the floor of the cage. During the first days, they hop along unconcernedly. After several days' rotation, they seek to rest. Most animals then lie lengthwise in the cage and try to slide along. This is not successful because the feet catch in the meshes of the floor. The animals now seem to become somewhat more irritable than was observed under normal

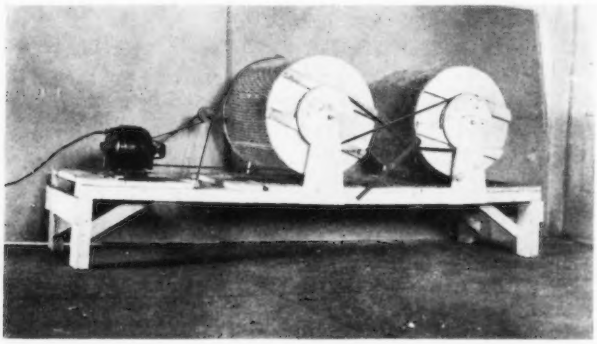

Fig. 1. Photograph of apparatus used for preventing sleep in the rabbit

conditions. Some animals roll on their sides or backs but they have to hold their heads up in order to keep them from being rubbed and irritated by the wire of the cage. This position and effort would seem to be quite fatiguing. We noticed that animals behaving in this manner reached the stage of exhaustion more quickly than animals which remained on their feet and hopped along throughout the experiment. However, it may be that the animals having the greatest stamina remained on their feet longer and withstood a more prolonged period of wakefulness.

Occasionally animals died during the night and in these cases the material was not used for histological study because of the possibility of post-mortem changes. All of the histological material examined was obtained from animals at the instant of death. In some cases both carotids of an animal in a state of collapse were severed and the tissues were re-

moved and placed in the fixative agent within ten minutes of cutting the arteries. The method employed for killing animals in experimental work of this type is very important. It must be as quick and painless as possible and it must as far as possible avoid the death struggle. Thus, cellular changes incident to violent muscular exercise are reduced to a minimum. The method seems better than death by anesthesia which often involves a prolonged period of excitement and muscular activity.

The fixation methods will be described in subsequent papers in which the histological phases of the work are to be reported. Likewise, the symptoms shown by the animals as a result of sleeplessness will be presented in the communication immediately following this.

SUMMARY

1. We have described a very simple apparatus for keeping animals (rabbits) awake continuously until complete exhaustion occurs. This was accomplished without subjecting the animal to excessive exercise. Such animals were then used for studying the histological changes in the tissues occurring as a result of loss of sleep unattended with any other factor, such as excessive muscular exercise, avoidable infection, etc. Later it is proposed to study in detail functional as well as biochemical changes, including power to resist infection and the effect of therapeutic agents on this type of exhaustion.

2. By the experimental methods employed, it is believed that objections raised to previous work in this field will be fully met and that trustworthy conclusions will be obtainable.

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STUDIES IN EXHAUSTION DUE TO LACK OF SLEEP

II. Symptomatology in Rabbits

CHAUNCEY LEAKE, J. A. GRAB AND M. J. SENN

From the Departments of Pharmacology and Anatomy of the University of Wisconsin Medical School

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A series of twenty male white rabbits was observed daily during the course of experimentation in the effort to produce exhaustion, according to the method previously outlined by Bast and Loevenhart (1927). Of this number, six were discarded because of high leucocyte count and infection noted shortly after the cages were set in continuous revolution, or because of diarrhea induced by the diet and possibly as a result of exhaustion. Observations were made for four or more days on the animals under normal conditions; that is, with the rabbits in the cages but without abnormal lighting, with the cages stationary, and with the same diet as when the cages were in revolution. A comparable series of seven animals maintained in similar circumstances except that the cages in which they were kept were never rotated, was also observed as controls, as previously described. These seven controls showed no significant symptomatology.

Receptacles were provided in the cages so that oats and water were always before the animals. During the run, the cages were stopped three times daily for the purpose of making observations, and in order to place small amounts of cabbage, alfalfa and carrots in the feed boxes accessible to the rabbits. It was found that under the conditions of the experiment the animals developed diarrhea when these fresh foods were constantly available, and therefore these foods were not kept in the cages continuously.

Observations of the respiratory and pulse rates of each animal were made at least thrice daily. In addition, daily observations were made of rectal temperature, urine output, the reaction of the urine to methyl-red, color of the mucous membranes, reaction of the pupils to light, character of the corneal reflex and knee jerk, and character of the feces. The rabbits were closely examined for sore spots on the feet or body which might have resulted from friction in slipping on the wire of the cages. If any such infected areas were found, they were at once cleaned and dressed. Soft leather boots were found to protect against infection of the feet and were provided, as a routine procedure.

Animals near the point of collapse were under observation almost continuously. They were removed from the cages and, with the cessation of respiration or pulse, were killed by bleeding from the jugular veins. The brain, spinal cord, thyroid and adrenals were promptly removed and fixed for microscopic examination, while the rest of the body was delivered to the Pathology Department of the University for further post-mortem study.

Since handling the animals caused excitement and change in the respiratory and pulse rates, as many observations as possible were made without removing the rabbit from the cage. Respirations could usually be counted while the cages were still in motion, since the speed of revolution was quite slow. The pulse was counted by stethoscope, leaving the animal in the cage after stopping rotation. Observations were made before feeding since eating usually increased both the pulse and respiratory rates.

The fourteen rabbits, on which satisfactory observations were made, lived from 8 to 31 days after the cages were put in continuous revolution. All these animals lost weight as the experiments progressed. For several days immediately after continuous rotation was started, the pulse rate of the animals increased, and the body temperature tended to rise. Whether this was due to excitement or augmented metabolism was not determined. Nine developed characteristic symptoms before death, while five were killed on the thirtieth day after the cages were put in motion without showing characteristic symptoms. The characteristic symptoms indicating approaching collapse and death seemed to be: *a*, a sudden fall in temperature; *b*, a rise in pulse rate above the level previously maintained followed by a sudden and marked fall, and *c*, a gradual fall in respiratory rate.

In two cases, after 7 and 17 days, respectively, in the revolving cages, the rabbits fell upon their sides when the cages were stopped. Muscular twitching followed and then the animals apparently went to sleep. In five or ten minutes, after a few convulsive movements, the animals resumed an upright posture, were alert, took food, and showed no further symptoms of fatigue. After being kept awake for more than a week, it was noted that many of the animals would immediately fall asleep and become perfectly relaxed if removed from the cage and placed upon a level surface. This sleep was so sound at times that the animals could be rolled over from side to side without waking them.

The symptoms of complete exhaustion were only observed in four animals, the others dying unexpectedly in the cages during the night when not under observation. In these exhausted animals, the body was completely relaxed but the muscles showed fibrillary contractions and the eyes were half opened. Marked salivation was observed and the mucous membranes of the mouth and nose were blanched. The corneal reflex was absent and the sphincters were relaxed. The pulse rate in these four

TABLE 1

Changes in body temperature, pulse rate, respiratory rate and body weight during experimental nervous exhaustion

Rabbit 7

DAY	BODY TEMPERATURE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	REMARKS
	°C.			
1	39.2	210	180	Normal Weight 2040 grams
2	39.1	216	190	Normal. Pupil 5 mm. in diameter
3	39.4	220	186	Normal
4	39.6	210	188	Rotation started on evening of this day
5	38.8	300	300	
6	40.1	300	180	
7	39.6	254	182	
9	39.5	272	188	
11	39.7	246	172	
12	38.8	238	168	Weight 1960 grams. Mucous membranes of nose and mouth pale
13	39.1	240	162	
15	39.4	236	158	
17	39.2	224	148	
19	38.8	220	136	
21	38.6	222	134	Weight 1900 grams. Mucous membranes of nose and mouth very pale
23	34.5	210	60	Weakness in legs. Falls soundly asleep when cage is stopped
25	36.1	306	66	Mucous membranes of nose and mouth very pink
26	36.1	276	48	Mucous membranes of nose and mouth pale. Feces soft
27	37.2	198	42	Weight 1810 grams. Animal seems very weak
28	36.0	240	100	
29(3:00 p.m.)	34.5	78	60	Both pulse and respiration irregular. The corneal reflex is sluggish. Mucous membranes of nose and mouth are blanched. The pupil is 1 cm. in diameter
29(3:45 p.m.)	34.5	132	60	Strychnine-like convulsions induced upon taking animal from cage. The mouth open. Marked salivation
29(3:50 p.m.)	34.5	48	10	Weight 1760 grams. Upon cessation of respiration, heart continues to beat feebly for 1 minute. Animal dies without convulsions

animals averaged 132 per minute, the respiratory rate averaged 42 per minute, and the rectal temperature averaged 35.6°C. Upon handling animals in this state of exhaustion, convulsions occurred. The head was thrown back, there was exophthalmos, and the front and rear limbs were fully extended. Following this convulsive seizure, the body completely relaxed, the pulse became irregular and feeble, the temperature dropped rapidly, and the respirations stopped. The heart continued to beat between one and two minutes after the respiration ceased.

It was impossible to predict, with any degree of certainty, the onset of complete collapse, inasmuch as the characteristic symptoms indicating approaching collapse were, in many cases, repeated several times before the animal finally collapsed and died. In these instances, the temperature, pulse rate and respiratory rate returned to the level generally maintained during the experiment, and after several days the syndrome would be repeated. There were no significant changes in the urine or feces, or in the response of the animal to electrical stimuli. A protocol for a typical animal is presented in table 1.

SUMMARY

1. Rabbits can be fatigued to a point of collapse and death by depriving them of sleep.

2. There is great individual variation in the endurance of rabbits to lack of sleep; the shortest period required to produce complete collapse was seven days and the longest was thirty-one days.

The characteristic symptoms indicating the approach of collapse and death are as follows: *a*, a sudden fall in temperature; *b*, a rise in pulse rate above the normal level followed by a sudden and marked fall, and *c*, a gradual fall in respiratory rate.

STUDIES IN EXPERIMENTAL EXHAUSTION DUE TO LACK OF SLEEP

III. Effect on the Nerve Cells of the Spinal Cord

THEODORE H. BAST, FREDERICK SCHACHT
AND HARRY VANDERKAMP

From the Departments of Anatomy and Pharmacology, University of Wisconsin

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Structure of the normal nerve cell. Even a superficial study of nervous tissue reveals the fact that there is a great variation in the different nerve cells. Malone (1913) in his extensive study of nerve cells has shown that even in a given cell type the individual cells differ as to size, structure of nucleus, protoplasmic content and size, sharpness of outline and staining reactions of Nissl bodies. He has shown that there is a marked difference in the nerve cells supplying striated muscle, those supplying cardiac muscle and those supplying smooth muscle. The characteristic structure which differentiates these three types of cells is the arrangement and size of the Nissl granules. He states that the cells of the visceral motor centres of the mammalian brain have a structure different from that of the cells of the somatic motor chain. The cells supplying heart muscle seem to take a position intermediate between those supplying striated and those supplying smooth muscle.

There is great variation in nerve cells depending largely upon the amount and distribution of Nissl substance. An accurate knowledge of the nature and distribution of this substance is paramount before we are justified in drawing conclusions regarding changes that occur in the Nissl content of nerve cells following various types of experimentation. According to Herrick, Mott and others Nissl bodies are fixation artifacts. The fact remains that these bodies, of whatever size or shape they may be, represent a definite substance in the living cells which we see in coagulated form. That this coagulation occurs in the exact location of the substance in the living cell would appear to be evident from the fact that it is present more or less constantly in all parts of the cell and its processes except the axone and the axone hillock.

The size, shape and arrangement of the Nissl bodies is subject to many variations. This variation may depend upon *1*, the conditions of the tissue at the time of fixation, *2*, the type of fixation used, *3*, the type of nerve cell studied and possibly *4*, the functional condition of the nerve cell.

Our experience here has shown definitely that the structure of the Nissl bodies depends in part at least upon the degree of autolysis which the cell has undergone in the interval between the time of death of the animal and the moment the tissue is placed in the fixative. If the tissue is allowed to remain in the animal 30 minutes after death and then fixed, the structure of the Nissl granules shows a marked variation from those in a tissue which was fixed within 10 minutes after the death of the animal. The type of fixative used also alters the size, shape and arrangement of the Nissl bodies. Cowdry (1924) states that mixtures of potassium bichromate and osmic acid do not give the sharp and clear pictures which may be obtained by using alcohol, mercuric chloride, acetic acid and other fixtures. Our experience has shown that an accurate and standardized histological technique is of paramount importance if the variations observed in the Nissl granules shall not be due chiefly to postmortem changes, fixation methods, and the stain used. Another factor which may cause variation in the Nissl granules is the functional state of the individual cell at the time of fixation. This is the problem with which this paper concerns itself. It is not the province of this paper to discuss the nature and significance of the Nissl material. For this the reader is referred to the work of Fleming (1882), Nissl (1885), Macallum (1891), Scott (1899), Hertwig (1902), Dolley (1909), Kappers (1921), Kulmatycki (1922), Nicholson (1923), and others.

THE STRUCTURE OF NERVE CELLS UNDER PATHOLOGICAL CONDITIONS. Many workers have attempted to show experimentally that the Nissl bodies are directly concerned in the functional activity of the cell. In chromatolysis following axone injury it has been quite conclusively shown that they undergo at first fragmentation and sometimes almost complete dissolution, but reappear again after the injury has been repaired. This finding led to the view that during nerve activity the Nissl bodies are used up and during rest they are replenished. All of the investigators who have studied the question experimentally have not reached the same conclusions. Most of them agree that in cases where the exhaustion was carried on to a sufficient degree, there is a change in the morphology of the nerve cell.

Kocher (1916) and Lambert (1893) were unable to find any substantial variation from the normal as to size of cell or nucleus, and their contents.

Hodge (1892), using animals in a condition of fatigue from ordinary exercise found that in the nerve cells of sparrows: *a*, there was a marked vacuolization in the cell protoplasm; *b*, the nucleus was oval and ragged instead of smooth and round; *c*, the nucleus was much shrunken; *d*, there was a lessened power of the cytoplasm to stain, and the nucleus seemed to stain just a little darker; *e*, occasionally there were found a few scattered fat granules.

The findings of Legendre and Pieron (1908) were similar to those of Hodge (1892). Nissl (1896) also found a decreased size of cell and nucleus,

but noted hyperchromatism as the chief result of fatigue. Vas (1892) and Mann (1895), on the contrary, found a decrease in chromatic material from exercise.

Perhaps the most extensive work has been contributed by Dolley (1909) and Crile (1921). In general they found that there is: *a*, an increase in the size of the cell; *b*, a variation in the relation between cytoplasm and nucleus; *c*, hyperchromatism is followed by hypochromatism.

Crile (1921) in his paper *Studies in Exhaustion* divides the process of fatigue into nine stages, the characteristic of the first stage being hyperchromatism and the final stage hypochromatism or practically achromatism.

Edmunds (1912) records the changes observed in the central nervous system resulting from thyro-parathyroidectomy. He performed his experiments on dogs and cats and noted changes in the cortex, cerebellum, cord and medulla. The changes in the medulla seemed to be the least marked.

A. Rendle Short (1919) describes changes in the central nervous system of three patients who died of shock. He states that the nerve cells of the sympathetic ganglia and the spinal cord showed no changes. The sensory nuclei of the brain (gracilus cuneateus and optic thalamus) showed profound loss of Nissl granules which he regarded as indicative of cell exhaustion. The motor nuclei (Betz cells, motor nuclei of the medulla and pons, lenticular nucleus) were normal. The Purkinje cells of the cerebellum, as Crile has pointed out, show considerable loss of Nissl granules.

The great variety of variation in the appearance of nerve cells in apparently normal animals suggests at once the danger of drawing erroneous conclusions from experimental animals unless the normal limits of variations are constantly kept in mind. Other variations in nerve cell appearance such as might result from postmortem change, fixation, variation of technique or death struggle resulting from poor methods of killing which might lead to erroneous conclusions have been stated in the first paper of this series.

METHOD AND RESULT. The methods of producing complete exhaustion by lack of sleep have been described in the first paper of this series.

The following table shows the number of animals used and the number from which our histological results were obtained.

	NUMBER OF ANIMALS	NUMBER DIED FROM EXHAUSTION	REMARKS
Series 1.....	15	9	One allowed to recover
Series 2.....	20	10	One animal that died from exhaustion was used immediately
Series 3.....	6	5	
Normal controls...	7		

The number of normal animals used was 7. The number of fatigued animals on which our results are based was 16. Animals which died were not used for histological study except in two instances in which the tissues were obtained the moment they died.

The symptomatology of these animals has been given previously.

HISTOLOGICAL PROCEDURE. After killing the animal by bleeding from the carotids the central nervous system was removed at once, cut into small blocks about 5 mm. in diameter and fixed in 95 per cent alcohol for 6 to 24 hours. They were then dehydrated in several changes of absolute alcohol for not less than 24 hours. The tissues were gradually brought into oil of cedar for clearing, and imbedded in paraffin. The paraffin treatment consisted of at least three changes of paraffin. Tissues remained in each change for at least 4 to 8 hours.

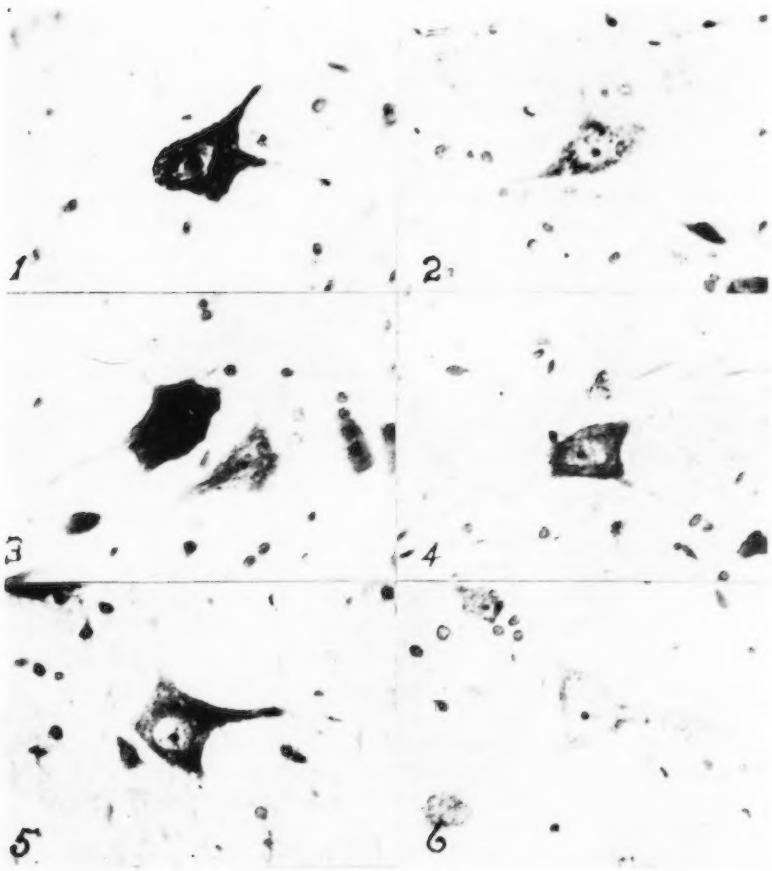
The sections were all cut in series at 6 micra and fixed on the slide with the water method.

The toluidine blue method was found the most satisfactory for our study. We used a $\frac{1}{2}$ per cent solution of toluidine blue, flooding the slide, heating until steaming and then allowing the stain to remain on the slide for 5 minutes. A uniform rate of staining and dehydration was observed throughout. The slides were mounted in balsam, examined under the microscope and cells showing characteristic changes were photomicrographed. Utmost care was taken to keep this technique uniform.

The problem of making a very thorough study of the normal cells and their extreme limit of variation was not a small one in our work. For this purpose seven healthy rabbits of approximately the same size and weight of our experimental animals and kept under identical conditions as the fatigued animals except that they were not kept awake, were killed by carotid hemorrhage and the various sections removed and fixed in 95 per cent alcohol. The same technique was employed in these controls as in the exhausted animals. Any variation in the staining resulting from a difference in technique was avoided by staining both fatigued and control section simultaneously.

HISTOLOGICAL OBSERVATIONS ON NERVE CELLS FROM CONTROL ANIMALS. In the normal cells a very definite variation could be observed especially between the cells of different size and type. While we did not go into a detailed study of the higher centers we noticed that variations were greater in the smaller cells of the cortex than in the large multipolar cells in the gray matter of the cord. The shape and size of the basophilic substance was quite variable but the outlines were sharply defined. The Nissl bodies occupied rather solidly the entire cytoplasm, except for the narrow clear areas between them. In the examination of the cells no chromatolysis or achromatosis could be found in any of the series of sections made from the control animals. We did notice, however, that the cells did not all take

the same density of stain, but even in such cases the Nissl bodies were well outlined. While there is a variation in size and shape of the cells and



These pictures are taken with a Spencer microscope 10 × ocular and a 4 mm. fluorite objective.

Figures 1 and 2. Normal nerve cells from control rabbits. Figure 1 contains much Nissl substance, while figure 2 has much less. In both cases the Nissl bodies are definite bodies.

Figures 3, 4, 5 and 6. Nerve cells from fatigued rabbits. The dark cell in figure 3 appears quite normal, but in the light cell the Nissl bodies are granular and vacuoles are present in the cytoplasm. In figures 4 and 5 the granular Nissl substance is quite diffuse except for the vacuolated zone midway between the cell wall and nucleus in figure 4. Figure 6 shows a cell in which the Nissl substance is very greatly depleted.

density of Nissl bodies in the somatic, motor, visceral motor, visceral sensory or the somatic sensory the basophilic substances whether large or small are definitely outlined in the various cells. See figures 1 and 2. The definite bodies, then, and not necessarily the density of the stain determine their normality. In a few rare cases a chromatolytic cell was seen.

HISTOLOGICAL OBSERVATIONS ON NERVE CELLS FROM EXHAUSTED ANIMALS. In every case where the rabbit had been carried to a stage of complete exhaustion and depression, certain cell bodies somewhere in the gray matter, usually in the visceral motor area, showed partial or complete chromatolysis. The majority of the cells, however, appeared normal especially in the somatic motor areas. In the animals which were not fatigued to the point of collapse it was practically impossible to detect a change from the normal control. In the accompanying charts the degree of chromatolysis is recorded for each animal.

The first sign of chromatolysis is a breaking up of the Nissl bodies into granules. In the early stages of this process the individual Nissl bodies can be recognized but they are granular. Then the granulation becomes more diffuse throughout the cell. This is followed by a vacuolization. Vacuoles first appear in a zone midway between the cell wall and the nucleus. The vacuoles increase in number until in severe cases most of the Nissl substance has disappeared.

Figures 1 and 2 show normal cells from a control animal. The first is a cell which has much Nissl substance and the second has very little. But in this latter the Nissl substance present is arranged into definite masses which are not granular. In figure 3, taken from fatigue animal 15, series II, is shown a cell with much Nissl substance which appears normal except in the dendrites, and another cell which has little Nissl substance and this is in a granular condition. A zone of vacuoles is also seen in this cell midway between the cell wall and the nucleus. Figures 4 and 5 taken from animals 5 and 15 respectively show the typical diffuse granular chromatolysis with the vacuolar zone. Figure 6 shows a cell with marked chromatolysis from rabbit 7.

We wish to emphasize, therefore, that our criteria for determining chromatolysis is not based on a decrease of Nissl substance but by: *a*, granulation of Nissl bodies; *b*, vacuolation of the cytoplasm. These two criteria seem to us to be the only reliable evidence for detecting chromatolysis.

The fact, however, that some of our animals which were not completely exhausted and a few that were, do not show marked chromatolysis, leads us to conclude that it is not as easy to detect nerve cell change following prolonged periods of sleeplessness as one might be led to expect judging from the findings of those who have studied the effect of other forms of fatigue on nerve cells.

Experimental results with special reference to the degree of chromatolysis in the cells of the spinal cord.

These results are based on a comparative study of the cells of the spinal cord from the following animals which were fatigued by loss of sleep as compared with the cells from the same areas of our control animals which were not fatigued.

NO. OF RABBIT	TIME RUN	CHROMATOLYSIS
	Series I	
	days	
1	6½	Moderate
3	14	Moderate
9	24	Moderate
11	8	Moderate
13	18	Moderate
	Series II	
3	11	Moderate
6	11	Slight
7	26	Very marked
9	9	Moderate
12*	56	Slight
15	31	Very marked
17	31	No marked change
18	31	Moderate
19	31	No marked change
20	31	No marked change
22	11	Moderate

* This animal had several extended periods of rest because the belt on the rotating apparatus slipped.

We have observed no shrinkage of the nucleus or cell as many workers report. In none of our preparations did we note a peripheral migration of the nucleus. The nucleus was always found centrally located. The normal nucleus usually takes a more or less homogeneous light stain. In fatigued animals the nuclei of nerve cells take little or no stain except for strands of chromatin radiating from the nucleolus.

Discussion. Our observations lead us to the conclusion that with extreme fatigue due to loss of sleep over a period of 7 to 31 days changes do occur in the nucleus and Nissl content of nerve cells. Since we find a great normal variation in the total Nissl content in different nerve cells we are convinced that a criterion for determining chromatolysis can not be based on the amount of Nissl material in a cell but rather on its structure and arrangement. The presence of only a small amount of Nissl substance does not assure us that it is the result of exhaustion, since we have no way to know the previous amount of the material in the cell in question.

We can find cells in all of our preparations from exhausted and control animals which contain very little Nissl substance. We can also find isolated cells which show chromatolysis in all of our preparations even in our normal animals. It is, therefore, very important to keep in mind these normal variations in determining whether or not actual change has followed a given type of experimental procedure.

Only in our animals fatigued to a point of collapse did we observe a definite nuclear change and chromatolysis according to our criteria in a sufficient number of these nerve cells to permit us to draw the definite conclusion that nerve cell changes occur as a result of fatigue.

SUMMARY

As a result of extreme exhaustion from lack of sleep the following histological changes are observed in the nerve cells of the spinal cord:

1. There is a decrease in the chromatin material of the nuclei.

2. Chromatolysis occurs and is characterized by: *a*, granular Nissl bodies or a diffuse granulation throughout the cytoplasm; *b*, the presence of vacuoles in the cytoplasm.

3. The vacuolation is usually found in a zone midway between the cell wall and the nucleus.

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STUDIES IN EXPERIMENTAL EXHAUSTION DUE TO LACK OF SLEEP

IV. Effects on the Nerve Cells in the Medulla

THEODORE H. BAST and WILLARD B. BLOEMENDAL

From the Departments of Anatomy and Pharmacology, University of Wisconsin

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The literature on the structure of nerve cells and the nature of Nissl bodies both in normal and fatigued animals has been cited in our earlier papers on "Studies in Experimental Nervous Exhaustion."

In reviewing this literature it is of especial interest in this connection to note that many of the investigators of changes in nerve cells as a result of fatigue find little or no change in the cells of the medulla of their experimental animals. Thus, Crile (1921) reports marked change in the nerve cells of the cortical region of his fatigued animals, but little or none in the medulla. He says he can offer no explanation for the localization of the histological changes in the cortex rather than the medulla.

Edmunds (1912) records the changes observed in the central nervous system in dogs and cats resulting from thyroparathyroidectomy. He noted changes in the cortex, cerebellum, cord and medulla. The changes in the medulla seemed least marked.

A. Rendle Short (1919) described changes in the central nervous system of three patients who died of shock. He found that the cells of the motor nuclei of the medulla and pons were normal.

All experimental evidence shows that the centers for the control of many of the most vital functions, such as: the respiratory vasomotor and vagal centers lie in the medulla. In the second communication of this series by Leake, Senn and Grab dealing with symptomatology, it was shown that these functions are severely disturbed in our animals which were exhausted by a loss of sleep.

The purpose of the investigation, therefore, was to determine whether or not there is any change in the nerve cells of the medulla in rabbits which were exhausted by depriving them of sleep. Not all of the medulla was chosen for this study but only the region in which lie those nuclei of the reticular formation in the region of the calamus scriptorius, nucleus hypoglossus, commissural nucleus of the solitarius, and the dorsal motor nucleus of the vagus.

The same animals and methods used in the study of the Nissl bodies of the spinal cord and presented in the previous paper were used in this study. The same technical methods were also employed.

Results. *Normal animals.* As in the spinal cord, so also in the medulla, did we find a considerable variation in the amount of Nissl material in the nerve cells of our control animals. In some the Nissl bodies were large and in others small. Some cells had little Nissl substance while others were densely packed with it. In all cases the Nissl bodies, whether present in large or small numbers, were definitely outlined and solidly staining bodies. Only in a few instances a cell was found in which the Nissl bodies appeared granular or fragmented.

The normal cells of the reticular formation and commissural nucleus are quite constantly packed with large Nissl bodies (figs. 4 and 5). The cells of the vagal nucleus contain fewer Nissl bodies scattered throughout the cytoplasm (fig. 1).

Animals exhausted by loss of sleep. The outstanding change from the normal medulla is the chromatolysis which certain of the cells show. The definitely outlined Nissl bodies of normal cells are absent. Instead the Nissl bodies appear fragmented or granular. In many such chromatolytic cells the Nissl bodies cannot be recognized as such and the chromatinic substance occurs in a granular form diffused throughout the cytoplasm. The chromatolysis seems to begin at the periphery. In certain cells in which chromatolysis is slight, the Nissl bodies surrounding the nucleus are quite normal in appearance, while those at the periphery are granular. As chromatolysis progresses all of the Nissl bodies become granular.

During the process of chromatolysis the cytoplasm also becomes vacuolated. The vacuolation is most marked in the area midway between the cell wall and the nucleus. Thus there are two zones of fragmented Nissl bodies separated by a clear vacuolated zone. Surrounding the outer zone of fragmented granules there is also another narrow zone of clear vacuolated cytoplasm (figs. 6 to 9). The extent of the chromatolysis is not the same in all cells of a given area. As a rule most cells in a given area are normal while some show moderate change and still others are almost completely achromatic. See figure 9. In some fields most of the cells showed signs of chromatolysis.

The nucleus which in normal cells stains a homogeneous light blue takes little or no stain in the chromatolytic cells. If some stainable material remains in the nucleus it is invariably found clustered about and radiating from the nucleolus. The nucleolus in all cases appears normal. See figures 5 to 9.

In addition to the above results, which were found to constitute the changes in the cells of the reticular formation and the commissural nucleus of the solitarius, we noted in the nucleus of the vagus in a number of cases

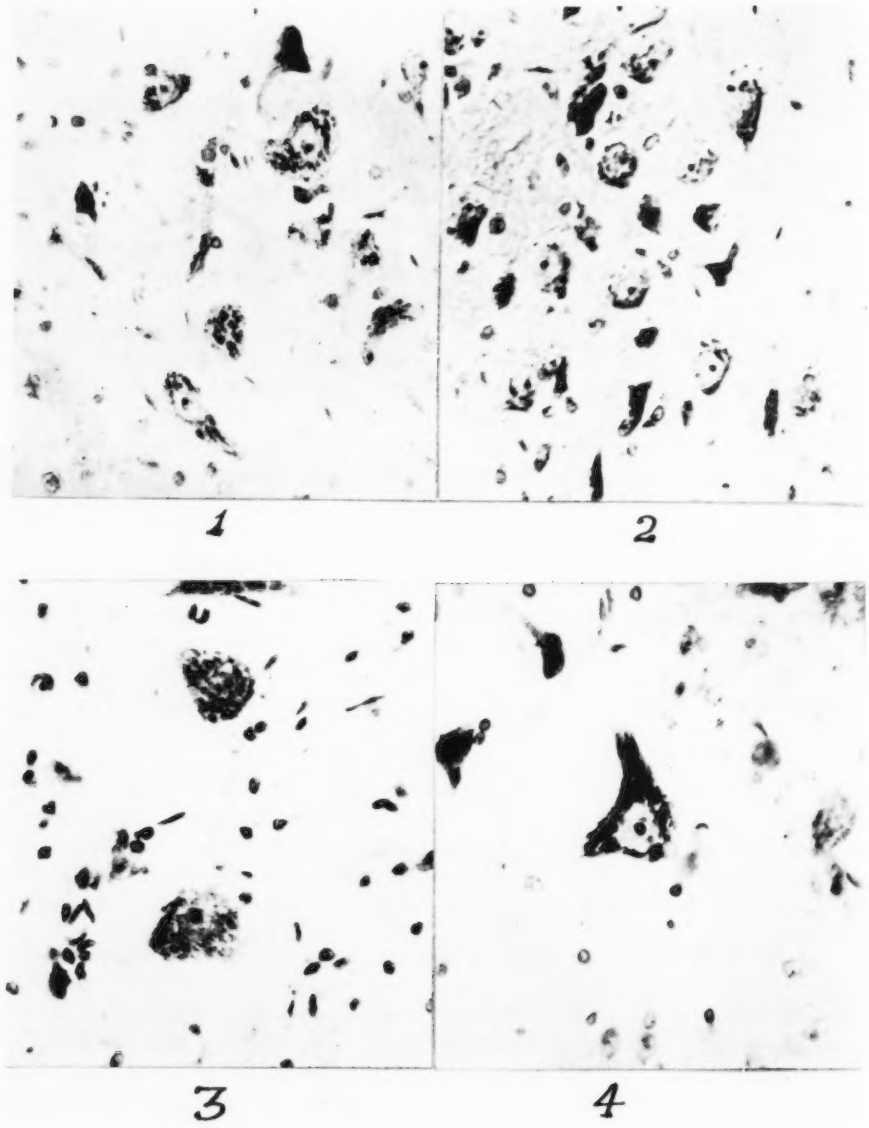

Fig. 1. A normal cell of the vagus nucleus. Normal rabbit 1.
Fig. 2. Cells of the vagus nucleus from exhausted rabbit 20.
Fig. 3. Cells of the commissural nucleus from exhausted rabbit 19.
Fig. 4. Normal cell of commissural nucleus. Normal rabbit 2.

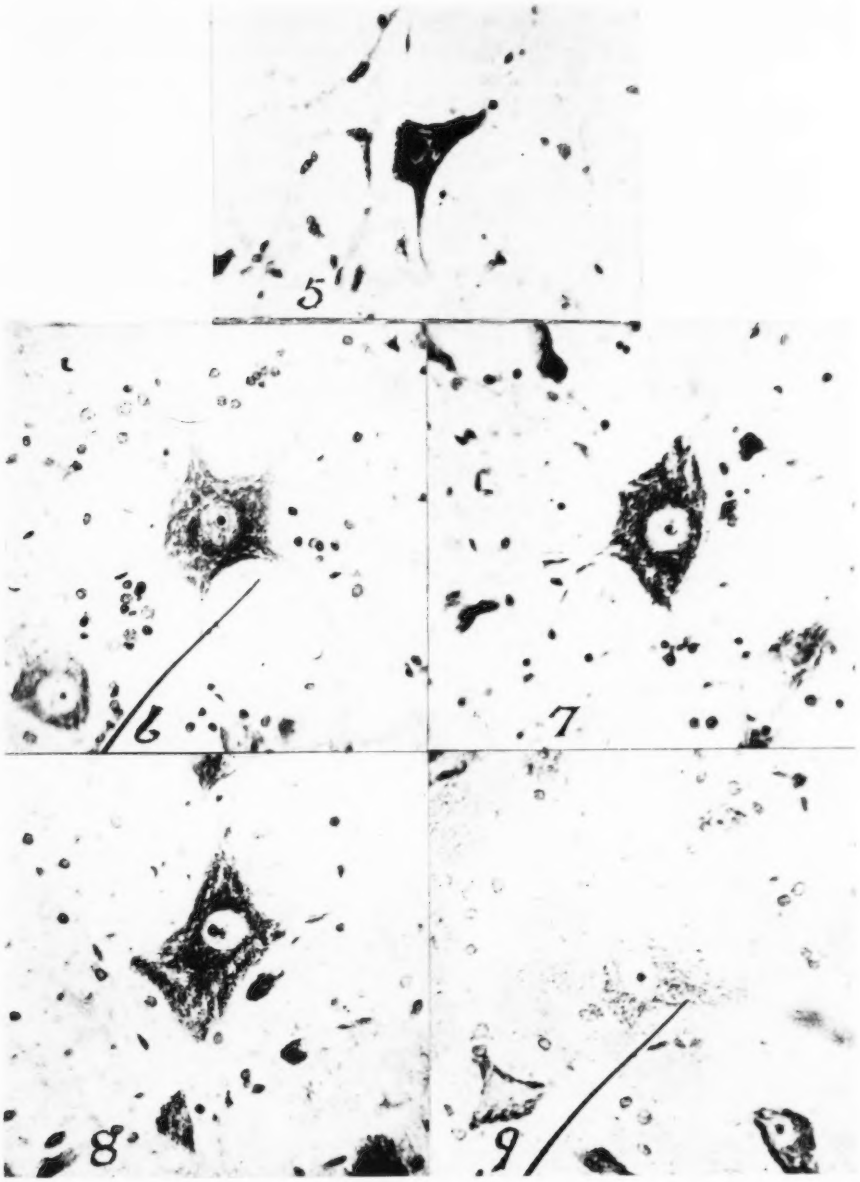

Fig. 5. Normal cell of the reticular formation. Normal rabbit 1.

Figs. 6, 7, 8 and 9. Cells in various stages of chromatolysis. They are cells of the reticular formation from exhausted rabbits 19, 6, 3 and 17 respectively.

that the cell walls appeared ruptured (fig. 2). This was not a constant finding. Crile (1921) and Hodge (1894) report this finding in their work on fatigued nerve cells. Hodge (1894) was at a loss to explain it, but Crile (1921) states that in fatigue there is an edema of the cell which produces the rupture.

The hypoglossal nucleus of exhausted animals showed no marked changes. In only one instance did we find evidence of chromatolysis and this was so slight and occurred in so few cells that it seemed to us to be of little importance.

The accompanying charts show the results obtained from the series of rabbits employed in this study. It shows the length of time required to

TABLE 1

Experimental results of animal series II, with special reference to the degree of chromatolysis in the cells of the medulla

These results are based on a comparative study of the cells of the spinal cord from the following animals which were fatigued by loss of sleep as compared with the cells from the same areas of our control animals which were not fatigued.

NUMBER OF RABBIT	TIME RUN	CHROMATOLYSIS			
		Nucleus ambiguus	Vagus nucleus	Hypoglossal nucleus	Commissural of sol. tarius
	days				
3	11	++++	+	−	+++
6	11	+++	+	−	++
7	26	+++	+++	−	+++
9	9	++	+++	−	+
12	56	+++	+++	+	++
15	31	+++	+++	−	++
17	31	+++++	++	−	+++
18	31	+++++	+	−	+++
19	31	++++	++		
20	31	++	+++	−	+++

exhaust each rabbit. Under "chromatolysis" we have indicated for each nucleus of the medulla studied the degree of the changes. One (+) indicates slight change from normal. Two (+ +) indicates a greater change from normal than one (+), and so on. Five (+ + + + +) indicates the greatest change that we have noticed.

Discussion. The criterion for determining chromatolysis is the same as that described in the preceding paper. In our exhausted rabbits the degree of chromatolysis in the cells of the medulla seemed more marked than in the cells of the spinal cord. In animals where little or no change was noted in the cord definite chromatolytic cells were found in the medulla.

It is also important to note that the somatic motor cells such as the cells of the somatic motor column of the anterior horn and the cells of the hypoglossal nucleus underwent little or no change as a result of exhaustion by sleeplessness. On the other hand it is exceedingly interesting that many of the visceral motor cells of the spinal cord as well as those of the medulla, namely, the cells of the reticular formation, nucleus ambiguus, commissural nucleus of the solitarius and the vagus nucleus showed changes. Although the degree of changes in cells of the reticular formation, the vagal nucleus and nucleus of the solitarius in different animals were not the same even though they had reached approximately the same degree of exhaustion, there was invariably a marked degree of chromatolysis.

Any explanation that we could advance at the present time for this difference between the visceral and somatic motor cells in their behavior under extreme exhaustion by loss of sleep would be too theoretical to be of great value. It will be necessary to have more detailed clinical observations and experimental results before we can determine the importance of this interesting difference.

SUMMARY

1. In our exhausted rabbits chromatolytic cells were constantly found in the reticular formation, commissural nucleus of the solitarius and the dorsal nucleus of the vagus.

2. Chromatolysis occurs and is characterized by:

a. Nissl bodies are granular.

b. Vacuoles appear in the cytoplasm. These are usually found in the zone midway between the nucleus and cell wall and sometimes in another zone at the periphery of the cytoplasm.

c. The nucleolus is normal.

d. The nucleus of the cells stains less deeply and the substance which does stain is clustered about and radiates from the nucleolus.

3. We have observed no migration of the nucleus.

4. The cells of hypoglossal nucleus showed no chromatolysis or other changes as a result of exhaustion by loss of sleep, and the same is true for all motor nerve cells supplying somatic musculature.

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A COMPARATIVE STUDY OF THE EXTENT OF THE KNEE-JERK AND THE ACHILLES-JERK

W. W. TUTTLE AND LEE EDWARD TRAVIS

From the Department of Physiology and the Psychopathic Hospital, State University of Iowa

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In this investigation the extent of the knee-jerk is compared with that of the Achilles-jerk. Apparatus has been devised which is capable of automatically delivering stimuli of uniform intensity, at a constant rate, to both the ligamentum patellae and the Achilles-tendon.

In case of the knee-jerk the subjects were seated in an adjustable chair so that they could be placed in proper proximity to a stimulating hammer. The apparatus used is essentially the same as that described elsewhere (Tuttle, 1924) except that the stimulating hammer is pulled back against a spring and automatically released instead of being elevated as a pendulum. Although the spring-propelled hammer strikes a sharper blow, more difficulty is experienced in keeping the strength of the stimuli uniform than is the case with the pendulum type.

The extent of the jerk is recorded by attaching the subject's leg to a stylus which writes on a smoked drum. It was found that the extent of the swing of the leg drew the stylus beyond the limit of the kymograph paper. This was avoided by placing a reducing pulley between the leg and the stylus so that their relationship is 1 to 3.75 mm.

Throughout the experiment a constant leverage was maintained by keeping the point of attachment of the recording device 30 cm. from the inferior margin of the patella. The stimuli were delivered at the rate of seven per minute.

A special apparatus was devised for eliciting and recording the Achilles-jerk. It consists of a revolving wheel, belted to a pulley of the power unit of the knee-jerk apparatus. A pin is placed in the side of the wheel which lifts a hammer and releases it again as the wheel continues to turn. By changing the fulcrum or by adjusting a movable weight on the hammer handle the strength of the stimuli may be varied. By increasing the number of pins in the wheel or by varying the relative size of the pulleys, the rate of stimulation may be varied. The unit is mounted on an adjustable stand so that it can be placed in proper proximity to the subject.

For recording the Achilles-jerk the subjects were placed in a prone posi-

tion upon a well-padded table equipped with leg supports so that they could not change their position thus varying the point of application of the stimuli. In this as in the knee-jerk experiment the right leg was used.

By means of this technique a sharp blow was delivered to the Achilles-tendon at the rate of ten per minute. Inasmuch as the Achilles-jerk can be satisfactorily elicited more rapidly than the knee-jerk, due to the fact that less time is required for the member involved to return to its original position, this rate was found to be more satisfactory than seven per minute as used in the case of the knee-jerk experiment.

The extent of the Achilles-jerk was measured by connecting the toe of the subject's shoe to a stylus which wrote on a smoked drum. This was done by means of an adjustable clamp so constructed that a constant leverage could be maintained. The point of attachment of the recording device was kept 26 cm. from the malleolus medialis. This measurement represents the hypothenuse of the angle formed by the attachment of the foot at the ankle joint.

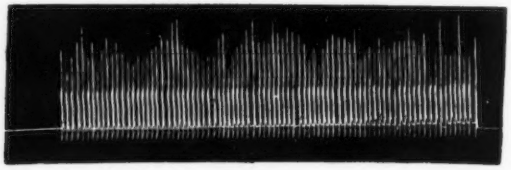

Fig. 1. A typical record of the Achilles-jerk

Experiments were carried out for the purpose of determining the reliability of the apparatus used for both the knee-jerk and Achilles-jerk experiments. The records obtained showed that the stimuli were delivered uniformly at the above mentioned rates and that the intensity of the stimuli was constant.

The movement of the toe was found to be well within the limits of the recording device used so that no reduction of the lateral movement was necessary. Thus in the case of the Achilles-jerk the record shows the actual distance moved.

The extent of both jerks is obtained by measuring the distance in millimeters through which the recording stylus moves. The readings for the extent of the knee-jerk are not corrected for the reduction.

Data were collected from 122 normal subjects. Of this number 60 were men and 62 were women. Each subject furnished about 70 knee-jerks and 100 Achilles-jerks for measurement.

The knee-jerk records obtained in this investigation showed no characteristics other than those described upon previous occasions. Since records

of the Achilles-jerk have not been previously shown, a typical example is given in figure 1.

Height, weight and age were obtained for each subject but it was found that there was no correlation between these factors and the extent of either jerk. It is evident that in the case of height and weight the size of the muscle groups involved is a compensatory factor.

The correlation between the strength of the two reflexes for 45 men is

TABLE 1

Means, standard deviations and ranges for the two jerks

	MEAN OF ACHILLES-JERKS	MEAN OF KNEE JERKS	S.D. dist. FOR ACHILLES-JERKS	S.D. dist. FOR KNEE-JERKS	RANGE OF ACHILLES-JERKS	RANGE OF KNEE-JERKS	NUMBER OF CASES
Women....	9.9*±0.40	33.7±1.3	4.77±0.30	15.8±0.96	0–19	4–86	62
Men.....	6.8 ±0.44	17.2±1.0	5.18±0.32	12.1±0.74	0–21	0–44	60

* All numbers in all tables refer to millimeters.

TABLE 2

Observed differences and their probable errors

	MEAN OF ACHILLES-JERKS	MEAN OF KNEE-JERKS
Women.....	9.9	33.7
Men.....	6.8	17.2
Difference.....	3.1±0.59	16.5±1.66

TABLE 3

Observed differences and their probable errors

	S.D. dist. FOR ACHILLES-JERKS	S.D. dist. FOR KNEE-JERKS
Women.....	4.77	15.8
Men.....	5.18	12.1
Difference.....	0.41±0.40	3.7±1.2

+0.37 ±0.12, for 54 women +0.54 ±0.09 and for 99 men and women +0.42 ±0.06.[1] Inasmuch as these correlations are not very large and have rather large probable errors there does not seem to be a very close correspondence between the strength of one jerk and that of the other for the same individual. This is readily seen when the individual records are

[1] Although 60 men and 62 women making a total of 122 subjects furnished data for most of the study only 99 could be used for correlation purposes because a number of the individuals lacked either one or the other or both of the two jerks.

studied. Subjects quite frequently give a vigorous knee-jerk and a weak Achilles-jerk and vice versa.

In comparing the irritability of men and women using the knee-jerk as an index, Williams (1925) found that the extent exhibited by the women was 42.08 per cent higher than that shown by the men.

The present study reveals a significant difference between men and women in regard to the extent of both reflexes, the women giving greater jerks in each case. This is shown in table 2 where it is seen that the difference between the average extent of the Achilles-jerk for men and the average extent of the Achilles-jerks for women is 3.1 $\pm$0.59 and the difference between the average extent of the knee-jerks for men and the average extent of the knee-jerks for women is 16.5 $\pm$1.66. Both of these

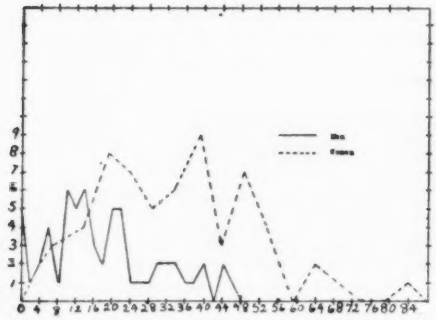

Fig. 2

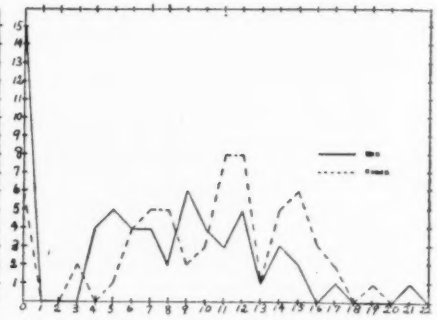

Fig. 3

Fig. 2. Distribution of knee-jerks for 60 men and 62 women. Ordinate gives the number of cases and the abscissae the extent in millimeters.

Fig. 3. Distribution of Achilles-jerks for 60 men and 62 women. Ordinate and abscissae same as for figure 2.

differences are sufficiently larger than their respective probable errors to make them highly significant. The averages show that knee-jerks of the men are about 0.5 as great as those of the women and that the Achilles-jerks for the men are about 0.7 as great as those for women. Also there is a genuine difference between men and women relative to the variability in extent of the knee-jerks. This is seen in table 3 where the difference between the $S.D._{dist.}$ for the knee-jerks of men and the $S.D._{dist.}$ for the knee-jerks of women is 3.7 $\pm$ 1.2. This difference is three times its probable error and consequently highly meaningful. Thus women as a group are more variable than men in regard to the extent of the knee-jerk. There seems to be no real difference between men and women in regard to the variability in the strength of the Achilles-jerks as the observed difference

in this instant (0.41) is not sufficiently larger than its probable error (0.40) to be of any significance.

The difference between men and women in regard to the extent of the two jerks is well brought out also by the distribution curves. These curves show that the women outnumber considerably the men at the upper levels.

The distribution curves indicate another fact, namely, that normal subjects fall into several rather well defined groups. For the Achilles-jerk

TABLE 4

Achilles-jerk groups for men and women

GROUP	HEIGHT OF JERK	NUMBER OF MEN	PERCENTAGE OF MEN	NUMBER OF WOMEN	PERCENTAGE OF WOMEN
A.....	0	15	25.0	6	9.7
B.....	3–8	19	31.6	19	30.5
C.....	9–13	19	31.7	20	32.3
D.....	14–21	7	11.2	17	27.5

TABLE 5

Knee-jerk groups for men

GROUP	HEIGHT OF JERK	NUMBER OF SUBJECTS	PERCENTAGE OF SUBJECTS
A.....	0	5	8.3
B.....	2–8	8	13.3
C.....	9–16	20	33.4
D.....	17–24	13	21.7
E.....	25–40	14	23.3

TABLE 6

Knee-jerk groups for women

GROUP	HEIGHT OF JERK	NUMBER OF SUBJECTS	PERCENTAGE OF SUBJECTS
A.....	4–29	28	46.8
B.....	30–44	18	29.0
C.....	45–59	11	17.8
D.....	60–90	4	6.4

men and women can be placed quite nicely in the same groups. This is shown in table 4. But for the knee-jerk the two sexes cannot be placed in the same divisions. Tables 5 and 6 give two sets of divisions for the knee-jerk, one for men and one for women.

It is interesting to note that with the strength of stimulus used 15 men and only 5 women showed no Achilles-jerk and 6 men and 0 women showed no knee-jerk.

SUMMARY

1. The correlation between the extent of the knee-jerk and the Achilles-jerk for 45 men is $+0.37 \pm 0.12$, for 54 women $+0.54 \pm 0.09$ and for 99 men and women $+0.42 \pm 0.06$.

2. The mean extent of the knee-jerks of 60 men was 0.5 as great as that of 62 women.

3. The mean extent of the Achilles-jerks of 60 men was 0.7 as great as that of 62 women.

4. The extents of the knee-jerk are more variable for women than men. The two groups show no difference in variation in extent of the Achilles-jerks.

5. For the Achilles-jerk men and women fall in four well-defined groups, the range of the groups being the same for both sexes.

6. In case of the knee-jerk men fall into five groups and women into four, the range of these groups being different for the two sexes.

7. Twenty-five per cent of the men and 9.7 per cent of the women had no Achilles-jerk.

8. Eight and three tenths per cent of the men and 0 per cent of the women had no knee-jerk.

9. There was no correlation between height, weight and age and the extent of either jerk for the group studied.

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SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD, ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

III. Urea

G. A. TALBERT, J. R. FINKLE AND S. S. KATSUKI

From the Physiological Laboratory of the University of North Dakota

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The excretion of urea from the skin has long been known, but like the study of the total nitrogen, the data that have been collected have been few and far between. Schottin (1) speaks of finding urea crystals in cases of uremia, which is of common knowledge today among medical men. Argutinsky (2) found that of the total nitrogen eliminated through the skin, 68.5 per cent was in the form of urea. Riggs (3) reports that the urea nitrogen varied inversely as the quantity of the sweat. Barney (4) reports that the percentage of urea nitrogen in the sweat tended to have a higher ratio than in the blood. Bleibtreu (5) observed a general increase of urea nitrogen eliminated as a result of sweat induced by mountain climbing. Marshall (6) in testing his urease method in two cases reports finding 0.014 and 0.00825 gram per 5 cc. of sweat respectively.

So much work has been reported upon the urea of the blood and the urine with their relationship to one another, that it hardly seems wise in this connection to make any citations. However, what relationship there might be between the excretion of urea through the skin and kidneys has been quite untouched. A still more virgin field presents itself in the simultaneous study of this constituent in the urine, sweat and blood.

In our experiments, we have discovered that the urea nitrogen of the sweat ranged from 0.24 to 1.12 mgm. per cc. The rather low concentration which we discovered in subject J. A. may be accounted for by his vegetarian diet.

In our determinations of the urea nitrogen of the urine, we found as low as 0.82 mgm. per cc., at the same time the sweat was only 0.07 mgm. per cc. On this occasion the subject had not partaken of any food for nearly 24 hours. The highest reading we observed was 27.6 mgm. per cc.

In the blood, the lowest urea nitrogen was 4.6 mgm. per 100 cc. in the subject L. M. The highest we discovered was 34.3 mgm. per 100 cc. on one occasion with subject F. C. It is a noteworthy fact that with this man it was either a feast or a famine, consequently the greatest variations

were in this subject. Table 1 shows the data obtained during the summer of 1925 in which F. C. was the principal subject. In table 2 in like manner

TABLE 1

Urea nitrogen in urine and sweat in milligrams per cubic centimeter (1925)

SUBJECT	URINE 1	URINE 2	URINE 3	URINE 4	SWEAT
F. C.	12.6		16.3		0.33
	13.5	21.0	12.9		0.56
	18.1	17.8	13.3		0.55
	6.12	15.7	8.0		0.52
	7.9	9.2	7.1		0.82
	9.9	17.2	11.4		1.70
	10.8	8.5	9.6	14.0	1.09
	8.9	11.5	10.7		0.92
	7.6	7.6	8.1		0.28
	7.0	6.9	8.9		0.32
	9.3	8.0	8.5	8.5	1.12
	4.9	3.9	3.0	5.3	0.39
	9.7	7.5	9.1	8.8	0.05
	16.6	14.1	12.1		0.59
	27.6	17.5	14.1		0.36
	4.4	1.6	0.82	1.16	0.07
	14.6	8.9	14.0		0.30
	8.29	13.0	10.2	13.3	0.24
	13.2	13.3	10.4		0.34
	8.4	8.9	8.4	7.3	0.40
	22.7	18.4	11.7	6.2	0.85
	20.8	19.2	19.1		0.66
	4.3	4.1	3.6	3.1	0.66
	11.5	12.9	12.7	10.4	0.54
C. H.	16.7	16.3	14.4	16.0	0.99
	13.3	12.4	17.6	14.9	0.56
	13.3	14.7	14.6	13.4	0.45
	10.7	10.0	10.7	9.7	0.40
	9.3	9.5	9.3	9.8	0.30
C. O.	13.5	13.5	12.9	9.6	0.66
	7.2	4.9	7.4		0.55
	7.8	8.1	11.6	8.2	0.53
R. T.	9.2	16.7	13.9		0.38

are shown the data for the summer of 1926 in which the blood tests are reported with that of the urine and the sweat. There are seventeen different subjects included in this table.

TABLE 2

Urea nitrogen in urine and sweat in milligrams per cubic centimeter. Urea nitrogen in blood in milligrams per 100 cubic centimeters (1926)

SUBJECT	URINE 1	URINE 2	URINE 3	URINE 4	SWEAT	BLOOD 1	BLOOD 2
H. I.	16.3	13.1	10.0			18.0	10.4
F. C.	11.5	16.4	16.6	15.0	0.87	12.8	5.7
C. H.	10.8	10.3	11.3	16.7	0.54	6.7	5.9
F. C.	13.8	12.5	8.8	7.5	0.47	18.8	18.2
W. G.	5.3	4.4	8.2		0.85	20.0	21.6
S. S.	9.1	8.5	7.9	10.5	0.32	15.0	16.2
W. G.	17.4	12.2	13.2		0.34	18.8	16.3
J. A.	8.9	9.9	9.1	12.1	0.59	19.0	18.4
F. C.	9.8	9.3	8.3	12.1	0.30	14.9	19.4
L. M.	10.3	9.4	8.0	9.0		5.0	4.6
F. C.		11.6	12.1	8.2	0.58	9.7	
J. Mc.	8.2	9.5	9.5		0.74		
F. C.	12.9	12.1	10.0	9.8	0.33	20.0	18.2
J. A.	3.1	5.4	5.1	4.0	0.34	17.5	20.0
F. G.	3.5	7.8	5.1	7.6	0.40	21.3	24.9
F. C.	11.1	7.4	5.5	7.7	0.43	17.3	17.0
G. Mc.	6.9	4.2	5.5	9.8	0.52	20.0	19.4
G. B.	13.3	10.4	10.2	17.4	0.43	16.5	10.0
F. C.	12.9	13.8	11.8	7.8	0.42	34.3	23.1
J. A.	2.8	4.7	4.5	3.7	0.45	8.1	5.9
I. H.	15.4	17.4	20.0	14.8	0.68	22.2	27.2
W. K.	4.5	4.2	3.7	3.3	0.43	15.0	12.2
I. R.	18.2	13.6	14.3	19.0	0.54	27.3	22.1
R. B.	18.2	16.3	7.9		0.61	19.9	11.8
C. H.		16.7	18.2			15.8	11.9
F. C.	11.7	9.8	9.9		0.42	14.5	9.6
J. A.	4.3	5.3	4.4		0.42	3.0	7.2
G. Mc.	8.7	10.0	9.1			6.8	13.3
W. K.	6.8	7.7	10.4		0.74	18.4	15.0
F. C.	17.9	12.9	16.7		0.50	4.5	16.2
I. R.	7.5	6.9	7.1			15.8	10.9
W. K.	10.5	15.4	14.3		0.89	8.6	20.0
C. H.	20.5	23.5	15.5			32.8	24.9
J. A.	9.1	8.6	13.8		0.41	6.6	15.0
I. R.	9.5	12.5	10.0		0.93	15.2	19.4
F. C.	21.1	22.2	21.5		0.41	25.0	20.0
O. G. H.	10.5	11.1	9.9		0.46	7.9	10.6
J. A.	15.2	18.2	22.2		0.40		
F. C.	18.2	19.0	18.2		0.36	14.6	15.0
W. K.	13.3	21.1	20.0		0.28	27.3	26.1
C. H.	15.4	12.5			0.31		
O. G. H.	9.1	8.0	8.3		0.42	13.6	7.1

SUMMARY

1. The minimum and maximum amount of urea nitrogen in the sweat, urine and blood are given.

2. Tables giving a complete report of the urea nitrogen in urine, sweat and blood.

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THE RECOVERY OF CONTRACTILITY AFTER CONTRACTION IN CARDIAC MUSCLE

HAROLD F. BLUM

From the Rudolph Spreckels Physiological Laboratory of the University of California

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In turtle heart muscle the amplitude of contraction alters with the rate of the contractions. An optimal frequency exists at which the contractions are maximal. If this frequency is increased or decreased from the optimum, the amplitude of the contractions decreases to a new level. These facts have been described for turtle atria by Smith (1926). Consideration of this phenomenon has suggested a study of the recovery of contractility after contraction in rhythmically stimulated muscles. Trendelenburg (1911) and Adrian (1920) have studied this recovery period quantitatively but not under controlled conditions of stimulation. In investigation of this subject the present writer has observed the phenomenon discussed below. It is believed that this has not been previously described.

METHOD. The muscle strips used in these experiments were cut from the apical border of turtle ventricles. These were suspended in the chamber diagrammed in figure 1, by means of stout thread tied around the two ends of the strip. Provision was made for the rapid removal and renewal of solution in the chamber at any time, and for passing oxygen through the solution.

It was found that the ventricular strips always developed more or less regular beats in ordinary Ringer's solution. Therefore a solution containing magnesium chloride similar to that described by Smith (1926) was used. Its composition was as follows:

NaCl	0.10 M
KCl	0.005 M
$CaCl_2$	0.002 M
$MgCl_2$	0.01 M
$NaHCO_3$	0.0005 M

The pH of this solution was about 8.0. In such a solution the strips remained quiescent or showed only rare contractions, while the contractility was not appreciably reduced in the course of several hours.

The tension produced by the muscle was recorded on a smoked drum by means of a tension lever similar to that described by Redfield and

Medearis (1926). The blade of a jeweler's hack-saw, supported in its own frame, served as the torsion spring. A very light clamp was attached firmly at the mid point of the spring. Projecting from the clamp, a stout steel wire about 1 cm. long served for the attachment of the muscle. This wire was bent into a small V with its apex just 1 cm. from the saw blade and directed downward. The thread from the muscle was attached at this point. On the opposite side of the saw blade, the clamp supported a short aluminum wire, horizontally and perpendicular to the blade. A very light hollow straw was fitted over this wire to serve as a writing lever. The writing point used was constructed after the method of Bayliss, described by Frank (1911). This consists of a celluloid point attached to a small square of heavy paper by a strip of goldbeater membrane. The celluloid point moves freely in a plane perpendicular to the surface of the smoked paper, but is quite rigid in a plane tangential to it. Thus a minimum of friction is obtained with a maximum of accuracy. The distance from the spring to the writing point was 17.7 cm., thus magnifying the shortening of the muscle 17.7 times. With a muscle 3 cm. long producing a record 3 cm. in amplitude, this represents a departure from "isometricity" of less than 6 per cent. The example cited represents a maximum deviation in the experiments performed, most of the contractions recorded approaching within less than 5 per cent of "isometricity." The natural period of vibration of the lever was 0.07 second. Since the contraction of the turtle ventricle muscle consumes over 2 seconds, the period of the lever is sufficiently high to eliminate any errors due to fling.

A signal magnet, marking the time of stimulation and a Jaquet chronometer marking seconds or fifths of a second, were also mounted to write on the drum. These, together with the tension lever, were supported on a Ludwig adjustable stand. An extra vertical rod was fastened to the rotating plate at the base of the stand, and the muscle chamber clamped to this. Thus the entire apparatus could be rotated horizontally to approach the smoked drum, the adjustable stop at the top of the Ludwig stand being set so that the pressure of the writing points against the smoked paper could be made constant in successive records. Also, the tension lever could be raised or lowered with regard to the muscle chamber by means of the vertical adjustment of the stand. This allowed for the adjustment of the initial tension on the muscle. The signal lever and chronometer maintained the same position relative to the lever during such adjustment, and it was thus possible to use the signal magnet as a reference point in the calibration of the lever.

The lower end of the muscle was attached by means of a stout thread to a hook at the end of a glass tube extending downward from the roof of the chamber (see fig. 1). The glass tube was fitted over an aluminum rod fastened securely in the roof of the chamber. The tube fitted closely

over the rod and was firmly attached to it by means of de Kotinsky wax. The rod could be bent slightly to adjust the position of the hook in the

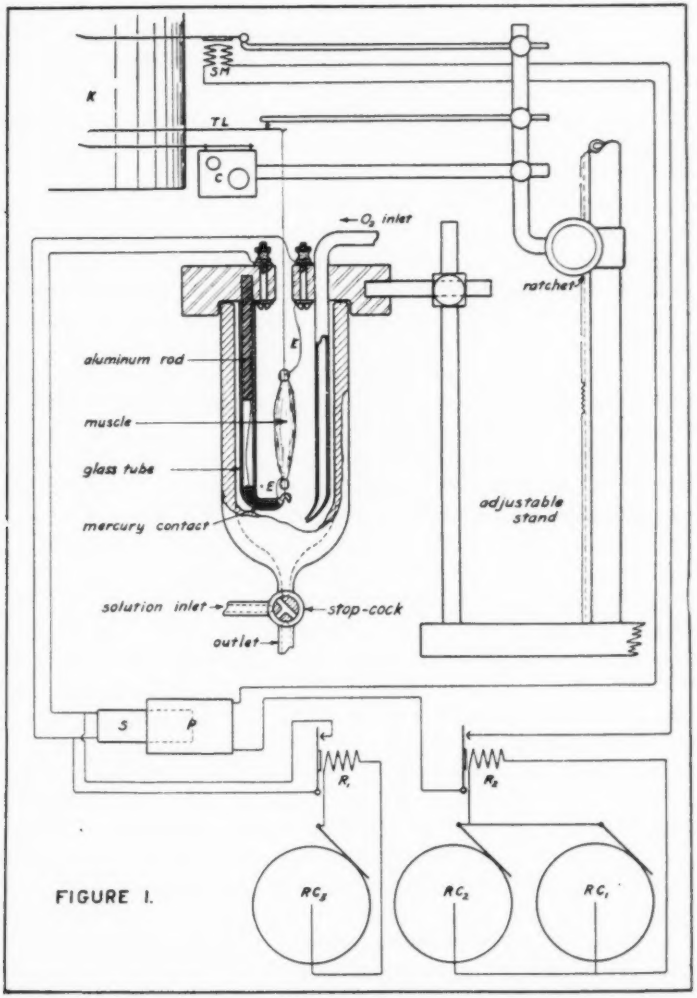


FIGURE 1.

Fig. 1. *S. M.*, signal magnet; *T. L.*, tension lever; *C*, chronometer; *K*, smoked drum; *E*, platinum electrodes; *P* and *S*, primary and secondary of inductorium; R_1, R_2, relays; RC_1, RC_2, RC_3, rotary contact makers.

chamber, but the scheme was sufficiently rigid to serve as a support for the muscle.

Calibration of the tension lever was accomplished as follows: A small paper pan of negligible weight was constructed so that it could be suspended by threads from the same point of attachment on the lever as was used for the muscle. Weights were placed on the pan, and the position of the lever corresponding to each load was marked on the drum by rotating it a few millimeters. The signal magnet served as a base line from which the distances corresponding to each load were measured. A calibration curve was prepared from these measurements and the records of muscular contraction analyzed in terms of this curve.

All measurements of these records were made to 0.1 mm. by means of vernier calipers. Since errors corresponding to 0.5 gram occurred occasionally in the calibration records accuracy beyond this point was not considered. Since the average tension production in the experiments was above 20 grams the greatest error is on the average 2.5 per cent. The maximum error in any experiment is not above 5.0 per cent.

Rhythmical electrical stimulation was provided by a rotary contact maker, operating a Porter inductorium through a relay. The relay assured uniformity in stimulation. One disk of the contact maker provided for shorting out of the "make" shocks so that only single break stimuli were used. Extra stimuli were sometimes provided by the operation of a telegraph key parallel to the contact maker in the relay circuit of the inductorium. In other cases, where exactly reproduceable intervals were required, one of three extra disks on the contact maker was used. These disks carried different numbers of contacts, and could be adjusted for various desired intervals after the rhythmical stimuli. An extra brush was constructed, which could be readily adjusted to any one of these disks, and was connected in parallel with the disk making the rhythmical stimuli.

The stimuli were carried to the muscle by two fine platinum wire electrodes. One of these was attached to a binding post in the roof of the chamber. The other was sealed into the glass tube supporting the muscle, a mercury contact being provided from which a wire was led out through the tube. The free ends of the platinum wires were pushed between the fibers of the muscle, one near each end. These wires were very flexible, and their tension on the muscle was constant and negligible.

Experimental results. In these experiments, the muscle was stimulated at regular, equal intervals, and allowed to reach a level of contraction (fatigue level). The interval used in establishing this level for any given series of determinations will be designated as the *basic interval* for that series. After any given contraction this interval was altered by interposing an extra stimulus before the basic interval had elapsed, or by prolonging this interval. For convenience in description, the contraction immediately preceding such an alteration of interval will be designated as contraction *1*. Those immediately following will be numbered successively *2* and *3*. Figure 2 is a schematic diagram explaining this numeration.

The graph shown in figure 3 was obtained from a series of such determinations on a single muscle. The interval between contractions *1* and *3* has been kept constant in this series. The curve representing contraction *2* indicates the progess of recovery of contractility following contraction at the basic interval of stimulation. The figure shows that if contraction *2* falls early (1.5 to 3 seconds) after *1*, contraction *3* is increased above the normal at the basic rhythm as represented by contraction *1*. Since the interval between *1* and *3* is kept equal to the basic interval,

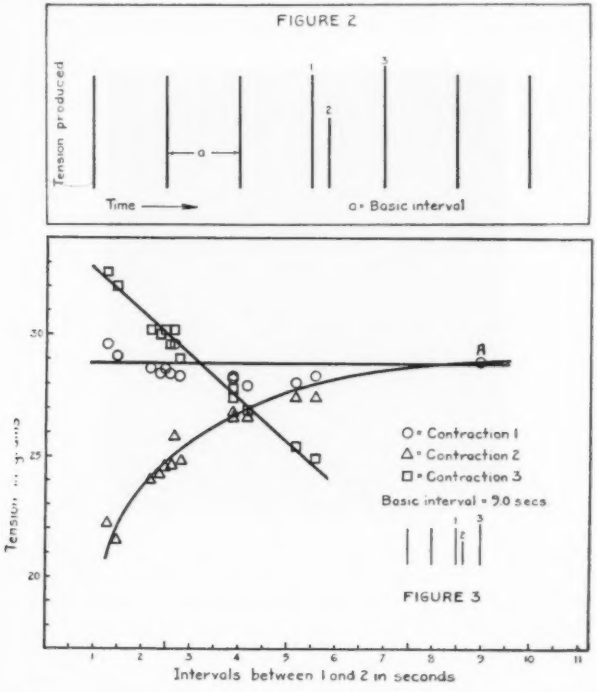


Fig. 3. Point *A* represents the average of a large number of determinations.

as the interval between *1* and *2* is varied that between *2* and *3* also varies in an inverse manner. Therefore, since the interval between *2* and *3* is actually less than the basic interval, the rate of recovery of contractility must be greatly increased following contraction *2*. In this figure, the curve representing contraction *3* falls below the normal level. This is obviously due to the decrease of the interval between *2* and *3* so that *3* falls within the relative refractory period of *2*.

Figure 4 is the kymograph record of a set of such contractions. This

record shows that the contractions following *3* become progressively smaller until, after a few beats, they again reach the basic level. This temporary alteration in contractility makes it necessary after each determination to stimulate at the basic interval for several beats before producing another alteration of interval. By doing this, each determination follows a period of contracting at the basic interval and the results are comparable.

Figure 5 represents a series of determinations in which the interval between contractions *2* and *3* has been maintained constant and equal to the basic interval. Under these conditions, the magnitude of contraction *3* may be taken as a measure of the rate of recovery of contractility.

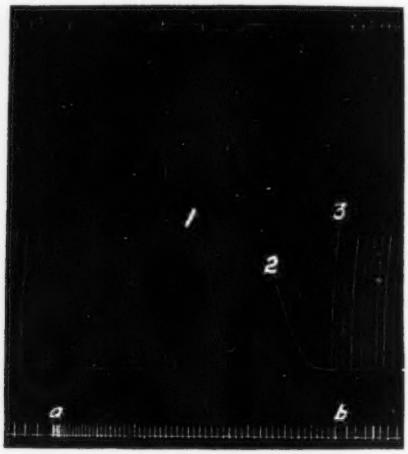


Fig. 4. Upper line—signal, marking time of stimulus. Middle line—myograph of isometric contraction of ventricle strip. Drum moving rapidly during contractions *1* and *2*. Remainder of contractions with drum stationary. Lower line—time signal, *a* to *b* fifths of a second. Basic interval 8.6 seconds, interval between *1* and *2*, 2.9 seconds. Note increased magnitude of contraction *3*.

From this figure it will be seen that the rate of recovery is greatly increased when contraction *2* occurs within a short interval (1.5 seconds) after *1*, but falls rapidly as this interval is increased. Obviously contraction *3* becomes equal to *1* and *2*, under the conditions of this series, when the interval between *1* and *2* is the same as the basic interval. As the interval between *1* and *2* is increased beyond this point, both contractions *2* and *3* are increased above *1*, contraction *2* being always greater than *3*. Both figures 3 and 5 are typical examples of numerous experiments performed on separate muscles under similar conditions.

Figure 6 represents an experiment in which the interval between *1* and

2 has been maintained constant (1.6 seconds) while the interval between *2* and *3* has been varied. Curve III represents contraction *3* in this series. For comparison, a curve obtained by varying the interval between *1* and *2*

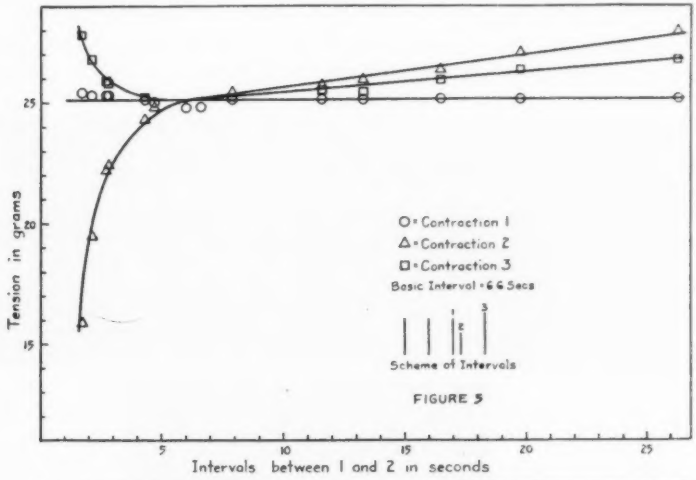


FIGURE 5

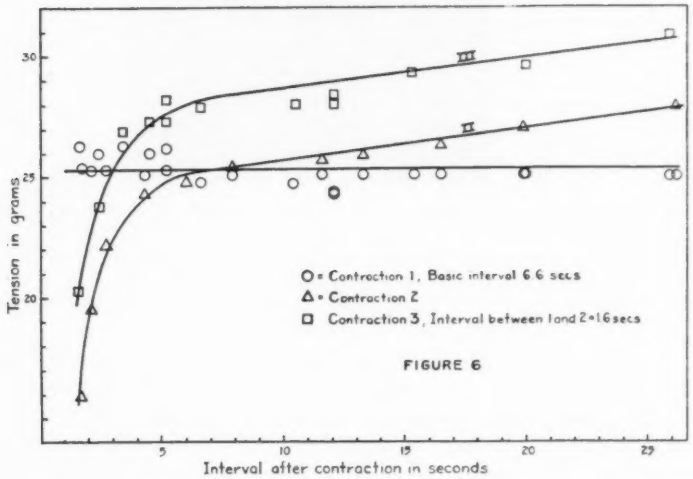
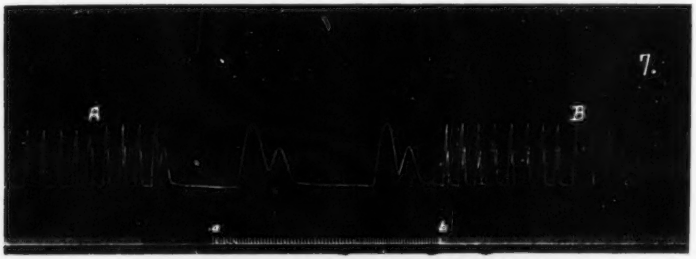


FIGURE 6

has been included on the same figure. This curve was obtained from the same muscle at the same basic interval. Curve II represents contraction *2* in this series. Curve III represents the recovery following a contraction 1.6 seconds after a basic contraction, while curve II represents the recovery

after a basic contraction. It will be noted that these curves are of the same type but differ quantitatively throughout. Curve III increases at a more rapid rate than curve II at first. It is probable that both approach asymptotically to the same value, although it is impossible to show this definitely with the data now at hand.

From the above experiments it seems justifiable to assume that the rate of recovery is markedly modified with the interval between stimuli.



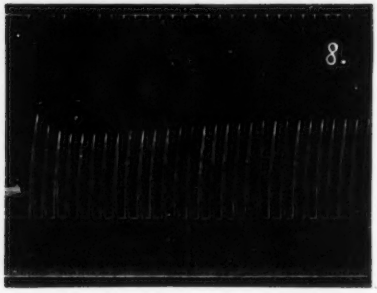


Fig. 7. Basic interval, 8.5 seconds *A* to *B*, extra stimuli interposed at 1.9 seconds after basic contractions. Note increased amplitude of basic contractions following *A* and decrease following *B*. Drum rotating at rapid speed during two sets of contractions. Lower line, *a* to *b*, fifths of a second, remainder seconds.

Fig. 8. Alteration in amplitude of contraction following period of rest (see text).

Figure 4 indicates that the alteration in contractility is very transitory in nature. Figure 7 is a kymograph record of a series of contractions in which after a period of contracting at a basic rhythm (interval 8.5 seconds) extra stimuli were interposed at short intervals (1.9 seconds) after the basic contractions. The height of the basic contractions is seen to follow a treppe, increasing to a new level which is maintained. When the extra stimuli are removed, these contractions are seen to fall rapidly to the original level. This is the case even when the extra contractions are maintained for a long period of time. From such evidence we may infer that the alteration of the rate of recovery is not due to the altered concentration

of any substance which cannot be rapidly removed. The rate of the recovery process seems to be very flexible and readily altered with the conditions of contraction.

It may possibly be objected that the phenomena described above are artefacts produced by the introduction of magnesium into the surrounding medium. It was found that although ventricle muscles always showed spontaneous beating in Ringer's solution, they remained quiescent in moist oxygen (see Lingle, 1905). Although the latter conditions were found unsatisfactory for prolonged experiments, it was found that results could be obtained, which agreed qualitatively with those described above. Similar results were obtained with muscles under nitrogen and carbon dioxide before the contractility was completely reduced. This indicates that the phenomena are fundamental in the contractile processes of heart muscle.

DISCUSSION. If the curve in figure 5 is carefully examined, it appears that the amplitude of contraction *2* varies as a function of the time interval between contractions *1* and *2*. Thus

$$a = ft$$

where a represents the magnitude of contraction *2*, and t, the time interval between *1* and *2*.

It appears also that the rate of recovery following contraction *2* as measured by contraction *3*, varies roughly as the reciprocal of the interval between *1* and *2* when this is less than the basic interval. This velocity reaches its minimum value at the basic interval, but as the interval is prolonged beyond this point, it appears to vary also with the amplitude of contraction *2*. It is thus possible that the velocity varies as a combined function of the amplitude of the preceding contraction, and the reciprocal of the time interval between the two preceding contractions. This may be expressed as:

$$v = \phi \frac{a}{t}$$

where v is the velocity of recovery following contraction *2*. But

$$a = ft$$

$$\therefore v = \phi \frac{ft}{t}$$

This becomes a rather complicated relationship which, however, is probably susceptible to mathematical analysis. Without further and more precise data, however, it does not seem justifiable to continue this analysis. It is hoped that this may be possible at a future date, with the accumulation of further measurements.

Inspection of curves of the type of figure 5 indicates that some such relationship exists. If we assume that the velocity v, at any given time, is dependent on the quantity of a substance V which is present, we may write:

$$V = \phi \frac{a}{t}$$

Let us assume that V is a substance produced at each contraction, in proportion to the magnitude thereof; but removed thereafter, very rapidly at first and approaching asymptotically to zero, or a constant quantity. In a series of contractions at a basic interval it follows, on this assumption, that after equilibrium is established, the quantity of V at the beginning of any contraction is the same. This follows, since each contraction is of the same amplitude and the interval between them is the same. We may call this quantity V_o. This should obviously vary with the basic interval used. When contraction *2* falls within the basic interval after *1*, a quantity of V greater than V_o is still present, to which is added that quantity produced by contraction *2*. Thus the velocity of recovery is greater than is normal for the given basic interval. When the interval between *1* and *2* is prolonged beyond the basic interval the quantity of V is reduced below V_o. However, the quantity of V added by contraction *2* is proportional to its amplitude and greater than that produced by a contraction at the basic interval. Thus the velocity is again increased above the normal.

Such an explanation fits at least qualitatively with the curves in figure 5, and the above mathematical expressions. This explanation also fits well with the observed conditions after periods of rest following periods of stimulation. Under these conditions the muscle always responds to the first stimulus by a large contraction followed by a relatively small one. The following few contractions may be progressively smaller, but will again increase in size in a long treppe. Such a series is shown in figure 8. This may be readily explained in terms of the above hypotheses as follows: During the period of rest, recovery has proceeded very nearly to the maximum, accounting for the large first contraction. The quantity of V is greatly reduced, however, accounting for the low rate of recovery following this contraction, and the smaller second contraction.

These suggestions can only be offered as a working hypothesis at this time with the hope that they may be developed and tested in the future.

Adrian (1920) described a supernormal phase of contractility in frogs' muscle perfused with Ringer's solution buffered to pH 6.4 to 6.6. This phase of hypercontractility occurs, according to his data, immediately following the relative refractory period, rises to a maximum and falls again slowly toward the normal level. He states that no such super-

normal phase occurs in alkaline solutions (above pH 8) but that under such conditions the contractility does not rise above the normal.

Neither of these events was observed in any of the experiments performed by the present writer. All of the latter experiments were carried on in alkaline solutions (pH between 8 and 9) but in all cases, the recovery continued to increase above the normal for long periods. Sometimes a decrease occurred, but only after intervals of five minutes or more. The results obtained with the turtle ventricle need not of necessity hold for the ventricular muscle of the frog. If the same phenomena occur in both cases, however, Adrian's results may possibly be explained in terms of the technique he employed. In his experiments, no fatigue level was established between determinations. The ventricle was rendered quiescent by ligation of the heart at the auriculo-ventricular junction, and paired stimuli immediately applied. The interval between the members of the pairs were accurately measured, but no uniform interval was employed between the pairs, which fall comparatively close together. If in frog ventricles, the magnitude of contraction is influenced by the interval between previous contractions, as in the case of the turtle, Adrian's results may have been confused by this phenomenon which he did not recognize.

According to the experiments performed by the present writer, a supernormal phase may occur, but only when the interval between contractions is prolonged beyond the basic interval at which the heart has been contracting. A supernormal recovery may be induced, however, by an altered interval between preceding contractions.

THE TREPPE PHENOMENON. The recent work of Smith (1926) shows that any explanation of the treppe in heart muscle on the basis of the direct effect of hydrogen ion concentration or lactic acid *per se* is not tenable. He has shown that changing the rate of stimulation of denoded turtle atria may cause either a treppe or an "inverted" treppe. Also, either of these phenomena may occur after short periods of rest, depending on the rate of stimulation before and after the rest period. These phenomena, which it seems difficult to distinguish from the classical treppe, occur in oxygen lack (when lactic acid is known to be increased), in solutions saturated with oxygen, or under high or low concentrations of carbon dioxide. Experiments by the present writer have confirmed these observations in the case of turtle ventricles. This seems to indicate that neither lactic acid *per se* nor hydrogen ion can be the determining factor in producing treppe, since the phenomenon takes place when these substances are present in widely varying concentrations.

Smith states, however, "The phenomena do suggest that there is some reactant in the muscle cell on the concentration of which, perhaps in some localized region, the development of tension is ultimately dependent, and that the concentration of this reactant is affected by the act of con-

traction itself." It is obvious that the extent of contraction depends upon the rate of recovery after a stimulus and the interval between the stimuli. Therefore, in order to accord with the results and hypothesis described above we may modify Smith's statement to read: there is some reactant . . . . upon the concentration of which the rate of recovery depends. With such a statement we may account for the phenomenon of treppe as a period during which the recovery rate is being gradually altered to accommodate to a new interval between stimuli. According to the hypotheses suggested, this represents the establishment of an equilibrium value for V_o.

Another factor may enter into this equilibrium. It is probable that a fatigue level is established in a rhythmically contracting muscle, at which the anaerobic production of lactic acid just balances its removal in oxidative recovery. An increased production of lactic acid may be accompanied by increased oxidative recovery (Meyerhof et al., 1925). Since H^+ ion (from lactic acid) may be one limiting factor to contractility (Redfield and Medearis, 1926) the adjustment of this balance must also be of importance in the production of treppe. It is impossible to determine, at present, whether this lactic acid balance is not intimately connected with the recovery rate described in this paper, but it is hoped that further investigation may help to elucidate this.

SUMMARY

The amplitude of contraction in heart muscle is dependent upon the rate of recovery and the interval between contractions. The rate of recovery is altered by alteration of the interval between preceding stimuli.

The velocity of the recovery process apparently is a combined function of the amplitude of the preceding contraction and the reciprocal of the interval between the two preceding contractions. An hypothesis is offered to explain the mechanism of this relationship.

The transient "supernormal" phase, described by Adrian for frog's ventricle in acid solutions, is not observed in turtle ventricle.

The alteration of the rate of recovery from one condition of stimulation to meet another, may be at least one factor in the production of the treppe phenomenon, and the establishment of the magnitude of contraction at a fatigue level.

The writer is much indebted to Prof. A. C. Refield of Harvard Medical School, who allowed him to use apparatus and materials for preliminary experiments, as well as unpublished manuscripts. It is a pleasure to acknowledge the kind advice of Prof. S. S. Maxwell, whose patience and encouragement have made the present work possible.

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THE REGULATION OF RESPIRATION

X. Effects of Carbon Dioxide, Sodium Bicarbonate and Sodium Carbonate on the Carotid and Femoral Flow of Blood

DETLEV W. BRONK and ROBERT GESELL

From the Department of Physiology, University of Michigan, Ann Arbor, Michigan

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A study of respiratory control is incomplete without a complementary study of circulatory control, for the respiratory and circulatory systems have a common function—dependent on a coördinated activity. Only by a systematic study of the performance of this common function under similarly controlled conditions can we arrive at a clearer picture of the mechanism providing a supply of oxygen and a removal of acid at the tissues. This seems all the more imperative with a theory of respiratory control involving as an essential factor the metabolism of the respiratory center itself (Gesell, 1923, 1925).

The importance of volume-flow for the maintenance of basal and superbasal metabolism of the body as a whole is firmly established (Krogh, 1922; Barcroft, 1914; Gesell, Foote and Capp, 1923; Gesell, 1919a, 1919b; Gesell and Moyle, 1922, and others. If we are willing to admit a similar dependence of the respiratory center on the volume-flow of blood (Gesell, 1923, 1925) circulatory disturbances in the medulla assume considerable importance. Take, as an isolated example, the effects of even a relatively slight hemorrhage. The total flow of blood may be sufficiently retarded to reduce the total oxygen consumption. Theoretically, a change in acid metabolism occurs with a decreased formation of carbon dioxide and a greater accumulation of fixed acid in the tissues. These changes which in themselves lead to increased acidity of the tissues, are augmented in their effects by a retarded flow of blood and a broken coördination of the dual function of hemoglobin. Both factors impair the transport of acid from the tissues. For a clue to the changes in acidity of the brain and body tissues as a whole under various conditions leading to altered ventilation, further studies on volume-flow are desirable.

For an understanding of changes in acidity of the blood a knowledge of the flow of blood is just as essential. In a preceding paper (Hertzman and Gesell, in press), it was shown that decreased flow of blood produced by hemorrhage leads to increased alkalinity of the arterial blood and increased acidity of the venous blood. Increased flow of blood from rein-

jection leads to increased acidity of the arterial blood and increased alkalinity of the venous blood. These changes occur whether the usual changes of pulmonary ventilation prevail or whether ventilation is maintained constant by artificial means. The effects of ventilation and volume-flow are thus clearly differentiated. It is therefore obvious that a change in blood acidity occurring in any acute procedure, hemorrhage, administration of nitrogen, cyanide, carbon dioxide, etc., which affects the volume-flow of blood, must in part be accounted for by this change in flow. As an example, the alkaline change occurring in the blood with the administration of air poor in oxygen is a resultant effect not only of increased pulmonary ventilation, which expels the carbon dioxide and the chemical change resulting from reduction of oxyhemoglobin, but to changes in volume-flow as well.

Granting a coördinated function of the respiratory and circulatory systems, and a dependence of functional behavior of the respiratory center on variations in its metabolism, it seems as logical to propose a control of circulation dependent on variations in metabolism of the circulatory centers. For just as surely as pulmonary ventilation influences the acidity on both sides of the neurone membrane, inside and outside the cell, so circulation influences these same chemical conditions. On the assumption that these acid relations are in turn significant in circulatory as well as respiratory control, the subject of circulatory control will be treated with that point in mind.

In the study of respiratory control continuous methods were used to follow the rapidly occurring changes during acute disturbances in equilibrium produced by hemorrhage, intravenous injection of sodium bicarbonate and carbonate, the administration of carbon dioxide, nitrogen, cyanide, etc. In these studies the probability of a coincidence of increased acidity and increased activity of the respiratory center was indicated and a frequent inverse relation between blood acidity and ventilation was established. That is, an increased alkalinity rather than an increased acidity of the blood is commonly associated with increased ventilation. These are relations which are more obvious and less easily missed in a study of rapidly changing equilibria than in a minute and accurate study of long-established conditions of equilibrium.

A study of the progress of acute disturbances in equilibrium would seem to offer the same advantages in the subject of circulatory control. It is a more difficult procedure to determine in absolute units by discontinuous methods the volume-flow of blood under changed conditions than it is to record changes in flow at the time of their occurrence. Perhaps that accounts for meager information on the volume-flow in man, which can be used to advantage to explain respiratory control. A small difference in flow obtained with discontinuous methods does not possess

the same value as the certainty of directional changes unfolded in the course of an experiment with changes in blood pressure, pulmonary ventilation and oxygen consumption simultaneously recorded. Another advantage of the continuous method in the acute experiment is the opportunity of following simultaneously changes in the brain and general circulation. After all, if the metabolism of the medullary centers is important in circulo-respiratory control, it may be as significant to know what is happening to the volume-flow in the brain as in the body as a whole. The danger in using the total flow of blood as an index to cerebral flow is apparent on recalling the reciprocal relation between cerebral and general flow with altered mental activity.

The difficulty of isolating the cerebral flow has been a hindrance to many problems. The continuous method, however, seemed to offer a crude approach for following changes in cerebral flow by recording the carotid flow. Though the carotid artery carries blood to skin, muscle, gland and bone, the cerebral flow may predominate. If, for example, a constant difference is noted in the carotid and femoral flows on the administration of carbon dioxide, it might not be unreasonable to attribute this difference to the effects of cerebral flow. On this assumption the carotid and femoral flows were recorded to follow changes in blood-flow distribution under a variety of conditions.

Two thermopile vessels were inserted: one in the carotid artery and one in the femoral artery. The flow was recorded as previously described (Gesell and Bronk, 1926). Blood pressure, ventilation, oxygen consumption and time were simultaneously registered. For the study of disturbance in the carbonate buffer system of the body, which was the purpose of this research, carbon dioxide was administered from rebreathing tanks with normal respiration and by artificial ventilation. Mechanical asphyxia was established by closing the valves of the tanks. Sodium bicarbonate and carbonate were administered intravenously.

Figures 1A and B show effects of mechanical asphyxia on the carotid flow of blood. Upstroke indicates increasing flow, the rate of which is designated in cubic centimeters per minute. Mean blood pressure, pulmonary ventilation and seconds and five-second intervals are recorded. The procedures are numbered at the base of the records. In figure 1A mechanical asphyxia lasts 50 seconds and in figure 1B 30 seconds. The effects are pronounced and exceedingly variable. The common effect is increased flow of blood, as illustrated in figure 1A. Diametrically opposite results appear in observations 1 and 3 of figure 1B, in which the flow is primarily decreased. In observations 2 and 4 the effects are quite different. The explanation of this alternating effect is to be found in the phase of respiration in which mechanical asphyxia is initiated. In observations 1 and 3 asphyxia began at the end of inspiration and in 2 and 4 at the end

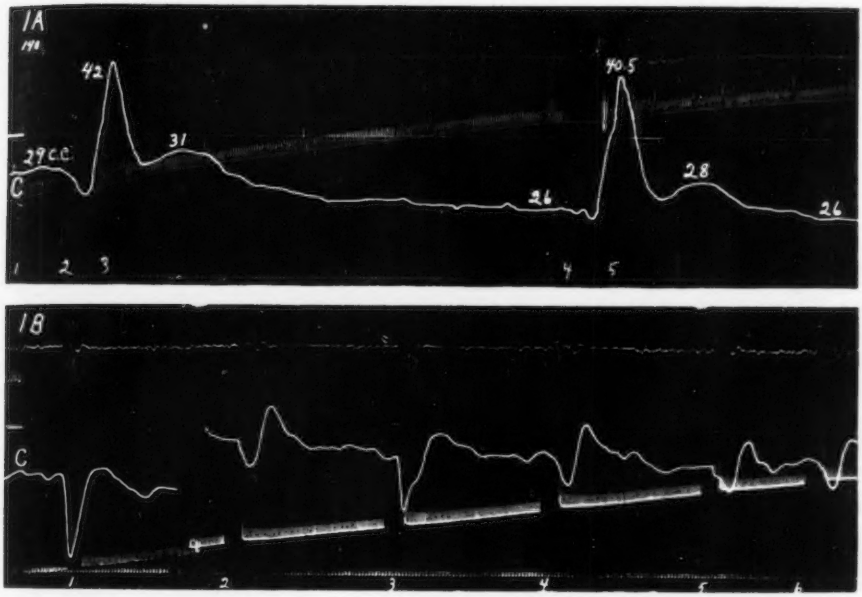

Fig. 1. Effects of mechanical asphyxia during normal ventilation with the chest intact on the carotid flow of blood. Mean blood pressure, pulmonary ventilation, carotid flow and time in second and six second intervals are recorded. The horizontal bar represents a period of 60 seconds. Upstroke indicates increasing volume-flow on the blood flow record and inspiration on the respiratory record. The first mechanical asphyxia of record 1A was initiated at the end of inspiration and the second at the end of expiration. Record 1B was taken from another animal. Mechanical asphyxias *1* and *3* were initiated at the end of inspiration; *2*, *4* and *6* at the end of expiration, and *5* at an intermediate point.

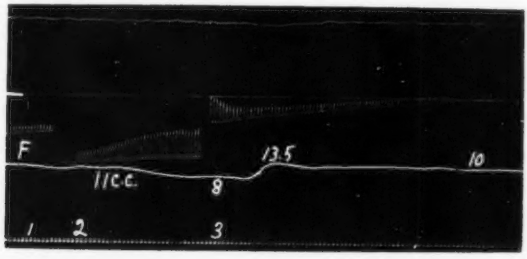

Fig. 2. Effects of administration of a 10 per cent carbon dioxide mixture in room air during normal ventilation on the femoral flow of blood.

of expiration. In figure 1A the moment of initiation of asphyxia also influences the results though not as markedly as in figure 1B. Apparently the effects of mechanical asphyxia are not entirely of chemical origin, and cannot be accounted for by changes in alveolar carbon dioxide tension alone. Mechanical and reflex phenomena are complicating factors.

The administration of carbon dioxide, therefore, seemed a simpler procedure for the study of the chemical regulation of volume-flow of blood involving the carbonate buffer system. The well-known effects of car-

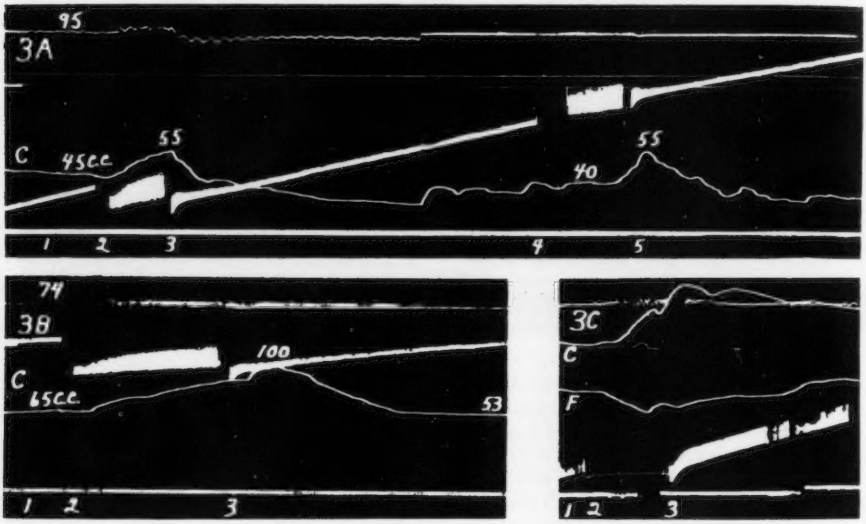

Fig. 3. Effects of administration of a 12 per cent carbon dioxide mixture in room air on the carotid and femoral flow of blood. In the second observation of record 3A and in record 3B a blood pressure regulator is connected with the aorta. In record 3C room air and carbon dioxide in room air are administered by artificial ventilation. Note the relatively constant mean blood pressure in this record. The pressure regulator was not connected.

bon dioxide appear in figure 2. The record shows a decrease in flow in the femoral artery from approximately 11 cc. per minute to 8 cc. resulting from the administration of a 10 per cent carbon dioxide mixture in room air. On readministration of room air the flow exceeds the normal and returns to a slightly subnormal flow. Since blood pressure remained remarkably constant the changes in flow seem primarily due to peripheral vasomotor action.

In figures 3A and B effects of carbon dioxide on the carotid flow and in figure 3C the simultaneous effects on the carotid and femoral flow are

shown. At *2* in figure 3A a 12 per cent mixture of carbon dioxide was administered for 135 seconds. The effects are the reverse of those just noted for the femoral flow. To rule out the increased flow resulting from

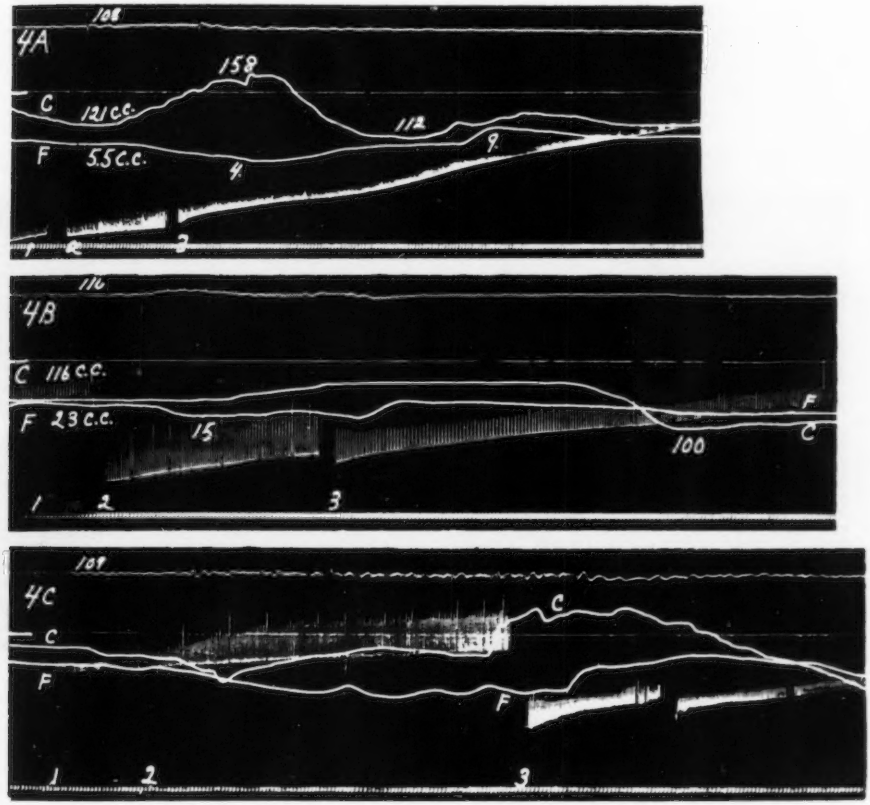

Fig. 4. Three records from three different animals showing the effects of temporary administration of carbon dioxide in the carotid and femoral flow of blood. The gases were administered by normal ventilation and the circulation was intact. In record 4A an 8 per cent mixture of carbon dioxide in room air was administered; in records 4B and C a 12 per cent mixture was administered.

passive dilatation from increased blood pressure, observations were made during a more constant mean blood pressure. A reservoir of blood suspended at a fixed height was connected with the lower end of the abdominal aorta. The second half of figure 1A shows results with the pressure

regulator connected. The irregularities of the record do not conceal the usual effects of carbon dioxide. Figure 3A taken from another experiment is a better example of flow changes during regulation of mean blood pressure. In figure 3C the pressure remains constant although no effort was made to keep it so. The usual results were obtained,—increased carotid and decreased femoral flow. Apparently the changes in flow are not due to changes in driving head of pressure. It is worthy of note that in figure 3C room air and the carbon dioxide mixtures were administered by artificial ventilation after the establishment of pneumo-thorax. Carbon dioxide is administered at *2* (ventilation not recorded).

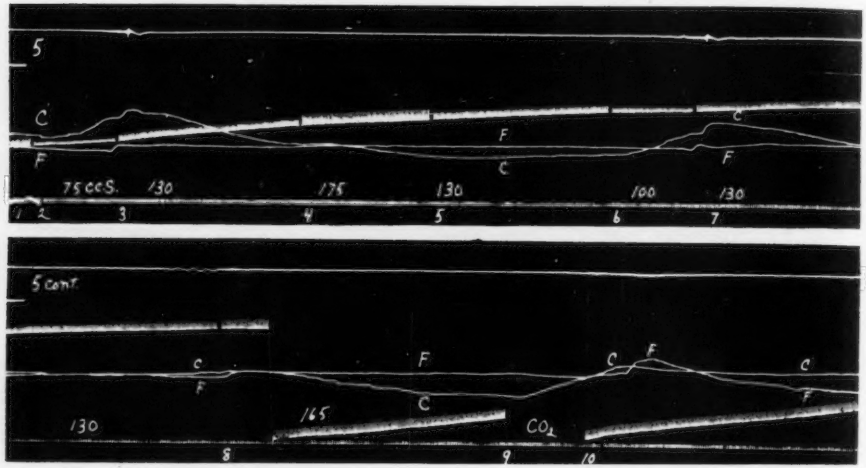

Fig. 5. Effects of variations in alveolar carbon dioxide pressure resulting from variations in artificial pulmonary ventilation produced by changing the stroke of the pump: *1*, 135 cc. stroke; *2*, 75 cc.; *3*, 130 cc.; *4*, 175 cc.; *5*, 130 cc.; *6*, 100 cc.; *7*, 130 cc.; *8*, 165 cc. At *9* carbon dioxide is administered. The directional changes in volume-flow on the administration of carbon dioxide agree with the changes produced by variations in pulmonary ventilation.

In figures 4A, B and C, results from three different animals are recorded. Typical effects of carbon dioxide are seen in the first part of figure 4A, but the nature of the later secondary oscillations in femoral and carotid flow is not so clear. It might be noted, however, that they are associated with variations in basal metabolism indicated by the respiratory record. Attention is called to the relatively small flow in the femoral artery as compared with the carotid flow.

In figure 4B typical results are obtained in the femoral flow. The increased carotid flow, however, is maintained much longer than usual and

curiously gives way in a sudden manner. This unusual behavior of the carotid flow was a constant finding appearing on three other trials in this particular experiment. The sudden decrease in flow was invariably associated with accelerated ventilation, as seen in figure 4B.

In figure 4C the femoral flow is typical but the carotid flow is somewhat irregular though the general directional changes are typical.

In the experiment shown in figure 5 the effects of variations in carbon-dioxide content of the blood and tissues are studied in another way.

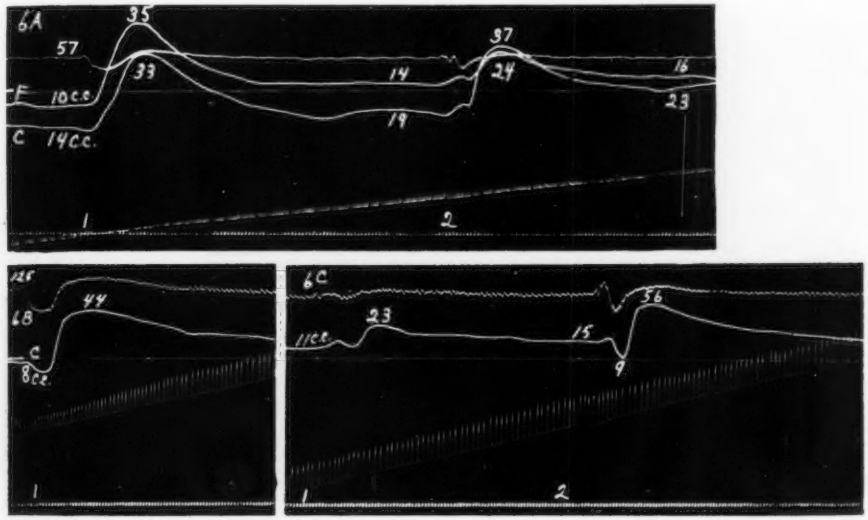

Fig. 6. Effects of intravenous injection of sodium carbonate, sodium bicarbonate and sodium chloride. In record 1A the effects of 20 cc. of M/2 Na_2CO_3 and 20 cc. M $NaHCO_3$ on the carotid and femoral flows are shown. In record 6B from another animal the effects of 20 cc. M/2 Na_2CO_3 on the carotid flow are shown. In record 6C 20 cc. of M NaCl and 20 cc. M $NaHCO_3$ were administered.

Instead of varying the alveolar carbon dioxide by administering the gas, the rate of removal of carbon dioxide normally formed is altered by varying the stroke of the artificial ventilation pump after establishing pneumothorax. The changes in carotid flow are easily followed as the flow was recorded with the sensitive setting of the potentiometer. The femoral flow which was recorded with the insensitive setting requires much closer inspection. It will be noted that a decrease in stroke leading to retention of carbon dioxide increased the carotid flow and decreased the femoral flow. Subsequent increased stroke decreased the carotid flow and increased the femoral flow. These results, which are repeated several

times, are finally checked at observation *9*, where carbon dioxide is administered followed by room air. The usual results are obtained.

The striking differences produced by intravenous injection of sodium carbonate and sodium bicarbonate on pulmonary ventilation suggested the study of the effects of these salts on volume-flow of blood. It would not be surprising to find opposite effects on volume-flow with the administration of these salts. The results can hardly be said to come within expectations (see figs. 6A, B and C and 7A and B). In the first place there was no constant difference between the carotid and femoral flow on

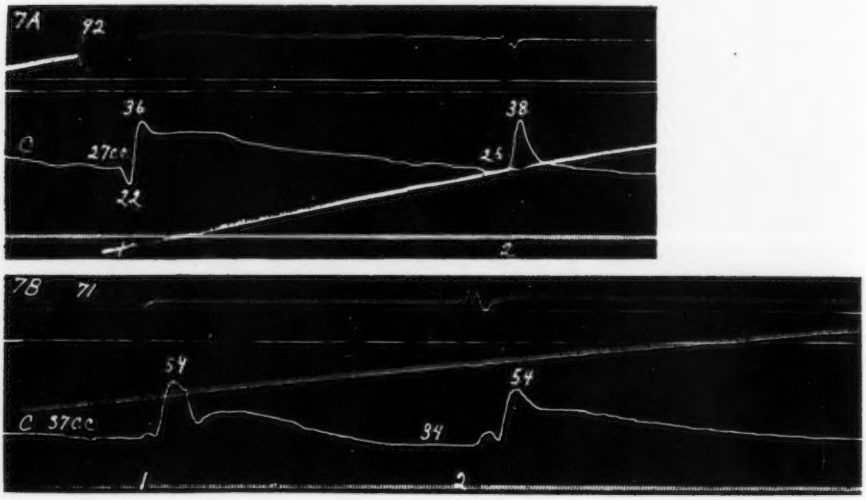

Fig. 7. Effects of administration of sodium carbonate and bicarbonate on the carotid flow of blood with and without regulation of mean blood pressure. In record 7A 20 cc. of M/2 Na_2CO_3 were injected first with the circulation intact, and second with the pressure regulator connected. In record 7B 20 cc. of M $NaHCO_3$ were injected first with the pressure regulator connected and second with the circulation intact.

the injection of either sodium bicarbonate or sodium carbonate. Sodium carbonate and sodium bicarbonate produced an increase in both. There were, however, minor differences in the effects of these salts. Sodium carbonate generally produced an initial fall in blood pressure usually associated with a decided drop in carotid and femoral flows. In observation *1* of figure 6A the initial decrease in volume-flow is virtually missing, which corresponds with the small accompanying fall in pressure. In figure 6B the drop in pressure is more abrupt and the initial fall in volume-flow is correspondingly large.

On the injection of sodium bicarbonate, which may elicit a prompt rise in blood pressure the volume-flow of blood is immediately increased and may at no time fall below normal (see fig. 6A). On the other hand—if the injection is rapid the initial increase in pressure may be followed by a drop below normal, accompanied by similar changes in volume-flow.

That the changes in volume-flow are related to change in blood pressure and blood-volume is indicated in figures 7A and B. In observation *1* of 7A sodium carbonate is administered with the circulation intact, and in observation *2* with the pressure regulator connected. In 7B the pressure regulator is connected in observation *1* and disconnected in observation *2*. The differences in the volume-flow records indicate that the initial decrease in flow is a result of a pressure change and the sustained increased flow to the combined effects of increased pressure and blood-volume. In figure 7B sodium bicarbonate is injected, first with the pressure regulator connected and second with it disconnected. It is interesting to note (see observation *1*, fig. 6C) that injection of sodium chloride is less effective than the injection of sodium carbonate or bicarbonate (see fig. 6C).

SUMMARY AND CONCLUSIONS

The effects of mechanical asphyxia, of the administration of carbon dioxide and the intravenous injection of sodium bicarbonate on the carotid and femoral flow of blood were studied with a continuous thermoelectric method.

Mechanical asphyxia elicited variable effects on the carotid flow. These variations were related to the phase of respiration during which mechanical asphyxia was initiated. It was, therefore, concluded that the changes in flow are attributable to mechanical and reflex effects as well as to chemical changes entailed in suspended ventilation.

To circumvent mechanical and reflex effects carbon dioxide was administered in room air by normal and by artificial ventilation. Such administrations increased the carotid flow and decreased the femoral flow. Subsequent administration of room air reversed these changes.

Variations in the magnitude of artificial ventilation produced changes in volume-flow of blood agreeing with those produced by the administration of carbon dioxide and room air. Decreased ventilation, which leads to accumulation of carbon dioxide in the blood and tissues, increased the carotid flow of blood and decreased the femoral flow. Increased ventilation produced the opposite effects.

It was demonstrated that the changes in flow were not dependent on changes in mean blood pressure.

The reciprocal relation between the carotid and femoral flow suggests that circulatory adjustments following variations in carbon dioxide content of the body occur in favor of the brain.

The opposite effects of sodium bicarbonate and carbonate on pulmonary ventilation were missing in volume-flow of blood. Both salts elicited an increased carotid and femoral flow of blood. No explanation is offered for the difference in behavior of these salts on the respiratory and circulatory systems.

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A THEORY OF MUSCLE CONTRACTION WITH X-RAY DIFFRACTION PATTERNS FROM RELAXED AND CONTRACTED MUSCLES

JANET HOWELL CLARK

From the Department of Physiological Hygiene, Johns Hopkins School of Hygiene and Public Health

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The work of Hill and Meyerhof has shown that, when a muscle is excited, there is a disappearance of glycogen and formation of lactic acid in equivalent amount. The liberation of lactic acid brings about the mechanical response, and relaxation is due to its prompt neutralization, probably by a potassium or sodium proteinate. In the phase of recovery some of the lactic acid is oxidized and the rest is synthesized back to glycogen.

There has been, as yet, no generally accepted explanation of how the lactic acid produces a mechanical shortening. Englemann's theory (1893) of imbibition in the doubly refracting bands is not supported by actual observations, for Hürthle (1909) finds no volume change in these bands on contraction. Recently there has been a tendency to ascribe the shortening to surface tension forces, but Hill (1925) finds this theory untenable. He calculates that the lactic acid liberated in a maximal contraction of a frog's muscle, if spread out in a continuous mono-molecular film, would occupy an area about equal to that of the surface of the fibres or to 2 per cent of the surface of the ultimate fibrils. If the mechanical response were due to a change in surface tension, caused by this film of lactic acid, the coefficient of surface tension would have to be 4800 dynes per centimeter to account for the force produced. This is about 230 times the tension of a water-olive-oil interface, and is clearly an impossible value, so it is evident that the shortening is not due to the surface tension of a lactic acid film.

Although no adequate explanation of the mechanical shortening has been offered, it has been the general opinion that the doubly refracting, or anisotropic, bands are the active agents in contraction. These bands are presumably composed of substances in a liquid crystal state; i.e., substances which show double refraction, while still in a liquid or plastic condition, owing to the fact that their molecules are arranged with the long axes all in the same direction. All substances that are capable of existing as liquid crystals show two sharp transition points at certain

definite temperatures where they pass suddenly from true crystals $\xrightarrow{T_1}$ liquid crystals $\xrightarrow{T_2}$ amorphous matter. Heat is given out when liquid crystals change to true crystals, and the change from crystal to anisotropic liquid is sharp and accompanied by a definite absorption of heat.

Extensive investigations of liquid crystals have been made by Lehmann (1904) (1921), Friedel (1922), Vorlaender (1908) and others, but so far the only substances that have been found to be anisotropic liquids or pastes at ordinary temperatures are soaps, such as sodium, potassium and ammonium oleate, lecithin, protagon, phrenosin and cholesterol oleate. These substances are all characterized by the presence of unsaturated fatty acids so that it is probable that the substance composing the dim or anisotropic bands in muscle fibrils is of a fat or lipoid character and contains unsaturated fatty acids. This substance might or might not be in combination with a protein.

THEORY OF MECHANICAL SHORTENING IN MUSCULAR CONTRACTION. The theory here proposed, to account for the mechanical shortening in muscular contraction, is that the substance in the anisotropic bands passes abruptly from a liquid crystal to a solid crystal form, as a result of the increase in acidity due to lactic acid formation. This hypothesis has already been stated in a preliminary communication (Clark, 1926).

A change in form from liquid to solid crystals in the anisotropic bands would bring about a molecular rearrangement, with closer packing of the molecules, and consequently a deformation, which would account for the mechanical shortening.

Besides accounting for the change in length on contraction, a change to crystal form, in the anisotropic bands, would produce a tension which can be very simply calculated, if certain assumptions are made, and which agrees rather well with the actual force in dynes developed by a contracting muscle.

When two similar plates are separated by a thin film of a liquid which wets them, an attraction occurs, and the force of cohesion between the two, arising from surface tension, $= \frac{2AT}{d}$ dynes, where A is the area of the plates, T is the surface tension of the liquid, and d is the thickness of the liquid film. When d is very small this force may be very great, as in the attraction between two glass plates, separated by a film of water. The same equation has been applied by Thompson (1916) to explain the tensile strength of metals. In this case two crystals, or layers of crystals, are separated by a layer of amorphous material, of thickness d, area A, and surface tension T, and the vectorial forces of crystallization must be postulated as the source of the attraction, instead of the atmospheric pressure which causes the attraction between glass plates separated by a water

film. If the equation is generally applicable to layers of crystals separated by thin layers of amorphous matter, and if we may suppose that increased acidity can change liquid crystals to solid crystals, then we have, in the contracted muscle fibril, alternate layers of crystals (anisotropic bands) and amorphous material (isotropic bands). This would be an ideal arrangement for the production of a tension, due to the cohesion between crystal layers, separated by thin layers of amorphous material, which can be calculated if the equation given above can be assumed to hold.

Hill (1925) has calculated that the sartorius of a frog can develop a maximum force of about 22,000 dynes per square millimeter. In this square millimeter there must be about 400 fibres, each 50 μ in diameter, so that each fibre must develop a force of about 55 dynes. The width of the isotropic bands is about 0.7 μ in a fibril 1 μ in diameter, which is the approximate diameter of the fibrils in the frog's sartorius. Therefore we know all the quantities in the equation except T, the surface tension of the substance of the isotropic bands. We may assume this to be 70 for the sake of convenience, since du Noüy (1922) gives 60 to 65 dynes per centimeter for the initial surface tension of fresh undiluted serum. The value of the force of cohesion developed in one fibre, as the result of the formation of crystal layers, can then be calculated.

$$\text{Force per fibre area} = \frac{2\,AT}{d} = \frac{2\,\pi\,r^2\,T}{d} = \frac{2\,\pi\,(0.0025\text{ cm.})^2 \times 70\text{ dynes per cm.}}{0.00007\text{ cm.}}$$

$$= 39.25\text{ dynes}$$

If the surface tension is taken as 60 dynes per centimeter, the force per fibre is 34 dynes. This is of the same order of magnitude as the value of 55 dynes given by Hill for the actual experimental value of the maximum force developed per fibre. This seems to indicate that, if a change to crystal form takes place on contraction, the change in form is the cause, not the result, of the tension.

THE EFFECT OF CHANGE IN HYDROGEN ION CONCENTRATION ON LIQUID CRYSTALS. This theory supposes that a change to crystal form takes place due to a change in hydrogen ion concentration. No mention is made in the literature of a change in crystalline condition with change in acidity, so before any attempt was made to prove the existence of crystals in contracted muscles, some preliminary experiments were performed on the effect of hydrogen ion concentration on the myelin forms of ammonium oleate.

Ammonium oleate is crystalline at -6°C. and shows a doubly refracting viscous phase from -6° to about 30°C., at which point it melts and goes into an amorphous state. Like all of the substances that are liquid crystals at ordinary temperatures, and which contain unsaturated fatty acids, it

gives rise to myelin forms in water. When a drop of ammonium oleate jelly is put in water at pH 7.6, the myelin forms, which are present in coiled form, begin to uncoil. An uncoiled thread, projecting free from the edge of the drop of jelly, has the appearance shown in figure 1, *a*. When examined in convergent polarized light, the coils are brilliantly colored and

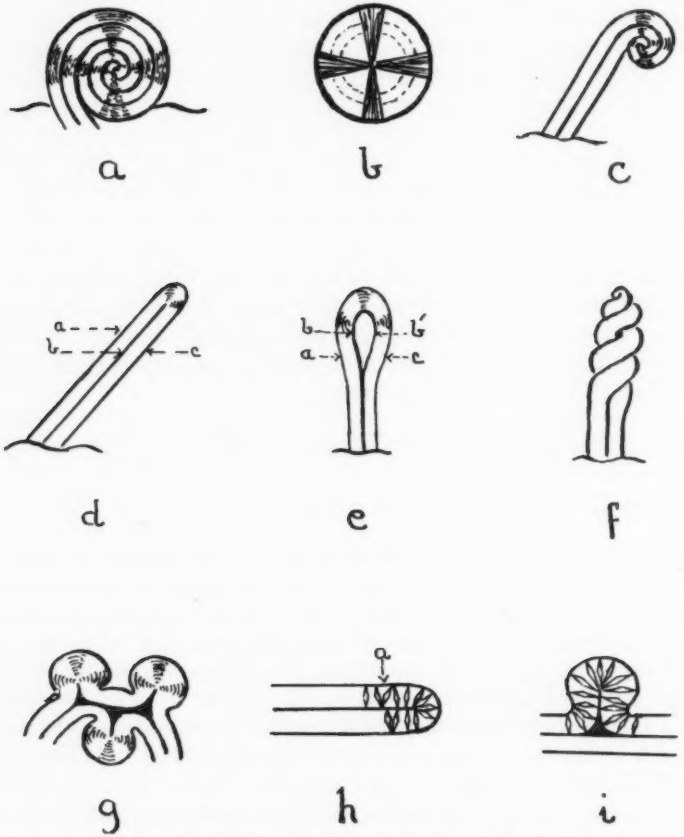


Fig. 1. Myelin forms of ammonium oleate in water.

the black cross, which is parallel to the directions of vibration of the Nicols, rotates with the analyzing Nicol, both with and without a $\frac{\lambda}{2}$ plate interposed. The cross pattern is similar to that given by spherulites, where acicular crystals are arranged radially (see fig. 1, *b*), whether the individual elongated crystals are uniaxial, with the length parallel to the

axis, or biaxial, with the length parallel to one of the three axes of the optical ellipsoid.

In water at pH 7.6 the coiled myelin form rapidly uncoils, probably due to imbibition of water, and presents the form shown in figure 1, *c*, with the cross still remaining in the coiled tip. It then straightens out completely and there is just a fragment of the cross left at the tip, where it bends on itself, giving a radial arrangement of molecules at this point (fig. 1, *d*). The myelin form behaves as though it were an anisotropic liquid enclosed between membranes *a*, *b* and *c* (figure 1, *d*), *b* being apparently a double membrane, for the myelin forms, on swelling, often show a large dark space between *b* and *b'* (see fig. 1, *e*). Frequently the coils are twisted on themselves giving a variety of shapes (fig. 1, *f*).

When a drop of water, acidified to pH 5.6 with dilute lactic acid, is added to the drop of water (pH 7.6) in which the myelin forms have extended, this very slight change in acidity produces a sudden contraction of the extended processes. Under low power they show a knotty appearance which, under high power, has the form shown in figure 1, *g*. In each knot the ammonium oleate has formed acicular cystals which arrange themselves radially producing spherulites in each protuberance. Acicular crystals are characteristic of a very rapid formation of crystals from a labile solution, when the crystallization is too rapid to permit the formation of well developed crystals which grow slowly. Myelin forms that are not knotted by small spherulites show clearly, under high power, the formation of elongated crystals of a bipyramid shape inside the myelin sheath (see fig. 1, *h*). Some of these elongated crystals are twinned, as at *a*, and if the twinning is sufficiently marked a spherulite is formed as in figure 1, *i*.

If the acidity is too great the protective myelin sheath, which is probably due to a concentration of certain molecules at the oleate-water interface, is disrupted and the crystals dissolve. There are thus three phases characteristic of ammonium oleate at room temperature, at different hydrogen ion concentrations. By placing the ammonium oleate jelly in buffer solutions the pH range for each phase was determined.

pH greater than 7.4: Extensive of myelin forms with a fluid anisotropic substance inside the myelin sheath, which imbibes water, and loses its anisotropic character if too much water is imbibed.

pH 7.0 to 7.4: Over this range there is extension of the myelin forms, due to the greater alkalinity at the surface of the ammonium oleate drop, but on reaching the buffer, where the pH is 7.0 to 7.4, the myelin forms retract with the formation of acicular crystals inside the sheath.

pH less than 7.0: The sheath breaks and the crystals dissolve.

Although the formation of small acicular crystals inside a fluid or plastic sheath can be followed visually under high power, at pH 7.0 to 7.4, these may be another modification of the liquid crystal form since Lehmann

(1921) reports the appearance of bipyramidal liquid crystals when ammonium oleate cools slowly from alcoholic solution. The probability that they are solid is, however, strengthened by the fact that a similar retraction of the myelin processes is produced by freezing the preparation on a salt and ice mixture. The appearance of the retracted forms is similar in pH 7.0 to 7.4 at ordinary temperatures and in pH > 7.6 at a temperature below 0°C. where the solid modification is known to occur. Also Rosenheim (1914) speaks of the following experiment with phrenosin. Phrenosin put in water, and gradually warmed, gives myelin processes. On cooling, these processes contract to about half their size and the contents are transformed into an aggregate of fine plates of the solid modification. If the water contains methylene blue, the processes that appear on warming are stained blue, but on passing back to the solid modification they change to a reddish violet. With ammonium oleate, in water at pH 7.6, the myelin processes stain blue in the presence of methylene blue, but when they retract, with the formation of small crystals inside the myelin sheath, they take on a pinkish hue.

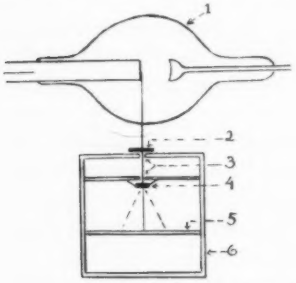
The evidence, therefore, seems to indicate that ammonium oleate is able to pass from the liquid crystal to the solid crystal form when the hydrogen ion concentration is slightly increased. Without going into the various possible explanations for this change, such as changes in surface tension, deimbibition, etc., the experiments were taken to indicate that the hypothesis stated above was at least plausible, even though they do not prove that a similar phenomenon occurs in muscle fibrils.

X-RAY DIFFRACTION PATTERNS FROM RELAXED AND CONTRACTED MUSCLES. There is an experimental method by means of which the nature and extent of crystallization in a substance can be determined, so that it should be possible to prove or disprove the hypothesis that crystallization takes place in the anisotropic bands on contraction. This method consists in obtaining x-ray diffraction patterns from the material in question.

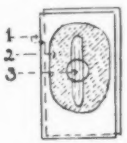
Method. The method used was the monochromatic pinhole method in which a narrow pencil of x-rays is isolated by two pinholes in lead sheets $\frac{3}{16}$ inch thick (see fig. 2 *a*). If a water-cooled molybdenum tube is used, and a zirconium filter is placed in front of the first pinhole, a monochromatic beam of x-rays is isolated with the wavelength 0.712 A. U. The specimen to be analyzed is placed behind the second pinhole and the x-rays, diffracted by the molecules of the specimen, fall on the photographic plate. The pinholes, specimen, and photographic plate are all enclosed in a lead box to cut off stray radiation.

The sartorius muscles of winter frogs were used throughout. They were surrounded by filter paper, moistened with Ringer's solution, and covered front and back with rectangular microscope cover glasses (see fig. 2 *b*) so that the muscle was practically in a moist chamber and was alive, and

apparently in good condition, after an exposure of six or seven hours. The cover glasses, with the muscle between them, were fastened to the under side of the second pinhole with adhesive tape, so that the monochromatic x-ray beam passed through the cover glases, which in themselves produced no diffraction patterns, and the muscle. The x-ray beam was perpendicular to the direction of the muscle fibres. The exposure time was from 5 to 8 hours, with a current of 5 milliamperes, and there was no difficulty in keeping the muscles alive and relaxed for this time by the method given above. It was, of course, impossible to stimulate the muscle electrically and keep it in a state of normal contraction for a long



(a)



(b)

Fig. 2

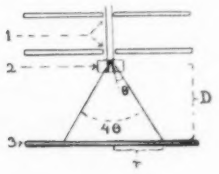


Fig. 3

Fig. 2. *a*. Apparatus for photographing x-ray diffraction patterns by the monochromatic pinhole method: *1*, water cooled molybdenum tube; *2*, zirconium filter; *3*, pinholes; *4*, muscle; *5*, photographic plate; *6*, lead box.

b. Arrangement of muscle *1*, rectangular cover glass enclosing *2*, moist filter paper with hole in centre, across which *3*, the muscle lies.

Fig. 3. Diagram of reflection from crystal planes: *1*, pinholes; *2*, muscle; *3*, photographic plate; *D*, distance between the muscle and the photographic plate; *r*, radius of ring or zone; θ, angle of reflection.

enough time, so that diffraction patterns for a contracted muscle were only obtained with muscles in death rigor and chloroform rigor. These contracted muscles were kept moist in the same way, since drying seemed to destroy the molecular orientation and no diffraction patterns were obtained with either relaxed or contracted muscles if they were dried at ordinary temperatures.

Different types of x-ray diffraction patterns. If a monochromatic x-ray beam falls on a large single crystal, the arrangement of atoms in a space lattice is so regular that the diffracted x-rays form a regular interference pattern of sharp spots around the central image.

In a specimen of microcrystalline structure, or in a crystalline powder the grains are arranged in an entirely chaotic manner. There are enough particles in this array, turned at the right angle to the incident beam of

monochromatic x-rays, to give strong reflection from one set of parallel planes. Other particles will produce reflection from another set of planes and the diffraction pattern will consist of a series of concentric rings, each uniformly intense, and each corresponding to one set of planes of spacing d.

There is also the condition in which an agglomeration of microcrystals approaches the condition of a single crystal by the alignment of the microcrystals so that the reflecting planes take up a common direction. When this occurs the concentric rings, instead of being uniform, are condensed into more or less well defined arcs or spots. This is known as fibre structure and is shown by hemp and cotton fibres, stretched rubber, worked paraffin and rolled metals.

There are two intermediate states between the amorphous and the crystalline condition, both corresponding to a certain type of liquid crystal. One of these is the nematic state in which the molecules are distributed at random but have one direction in common. The other is the smectic state in which the molecules have a direction in common and are also arranged in equidistant parallel planes, the distance between these planes corresponding to the length or width of the molecules, or to the length or width of a pair of molecules. The nematic anisotropic liquids do not give x-ray diffraction patterns, but the periodic position of the molecules in a smectic liquid crystal diffracts the x-rays like the parallel planes in a crystal. Consequently smectic substances, such as the oleates of ammonium, sodium and potassium, give a series of zones or hazy rings which are the first, second, and third orders of reflection from a set of equidistant planes. De Broglie and Friedel (1923) using monochromatic K-radiation from copper, found that the distance of these equidistant planes in sodium oleate was 43.5 A.U. This distance corresponds to twice the length of the molecule.

Liquid crystals of the smectic type, which are to be expected in the anisotropic bands of muscles, should, therefore, give a series of broad hazy rings. If there is microcrystalline structure in the muscles one should get sharp interference rings and, if microcrystals are suspended in a smectic fluid, there would be sharp crystal interference rings superimposed on the wide hazy rings of liquid crystals.

Diffraction patterns with frog muscles. Herzog and Jancke (1921) obtained diffraction patterns with hair, tendons and muscles. The fresh muscle showed two symmetrical spots in a hazy circular zone. When the muscle was stretched and dried the spots were clearer and the hazy zone sharper. Since it is evident from these experiments that some of the substances in muscles are capable of giving diffraction patterns, experiments were carried out on the sartorius muscle of the frog, in relaxed and contracted condition, to see if any change in crystalline condition takes place on contraction.

The photographic plate was first placed at a distance of 3.3 cm. from the muscle. After a five-hour exposure the plates showed two zones, the inner one very black and the outer one faint. Figure 4 shows these zones for the relaxed muscle (*a* and *c*) and for the muscle in death rigor or chloroform rigor (*b* and *d*). In *a* and *b* the second pinhole, which in this case was in a brass plate, was smaller than in *c* and *d* and consequently the second zones in *a* and *b* are reduced in intensity. The faint narrow ring on one side of photographs *a* and *b*, outside the zones, is caused by the brass in which the second pinhole was made and may be regarded as an artefact.

When the photographic plate was put at a distance of 5.7 cm. from the muscle the diameter of the two zones, shown in the photographs in figure 4, increased so that they appeared as hazy rings, instead of zones, and there was in addition a new zone close to the central spot. This new zone is the first order reflection, and the two zones shown in figure 4 (*c* and *d*) are the second and third order reflections, from a set of equidistant planes of oriented molecules. These hazy rings or zones are characteristic of liquid crystals in the smectic state.

When the diffraction patterns of the relaxed and contracted muscles are compared two differences are found but as the photographs are not retouched these differences may not show clearly in the reproduction. The first difference is that, in the relaxed muscle, the zones are of practically even intensity (fig. 4, *a* and *c*), while in the contracted muscle there is a distinct condensation into a ring at the edge of the zones. This effect shows better in comparing *a* and *b*, in which only the first zone (2nd order reflection) shows clearly. Here the even darkening of the first zone in *a* changes on contraction to a faint darkening near the central spot with a distinct ring at the edge of the first zone in *b*. This is not a clear-cut proof of the presence of solid crystals in the contracted muscle but suggests the presence of microcrystals suspended in a smectic fluid. Such a large part of the muscle substance is amorphous that it might not be possible to obtain a clearly crystalline diffraction pattern, even if all the material of the anisotropic bands went into the solid modification, and such change as there is suggests a more solid modification.

The other change on contraction is a change in the diameters of the rings or zones. This is more noticeable in figure 4 (*c* and *d*), where the second zone is clearly visible, although it was a consistent result throughout. In the contracted condition the diameters are larger which means that the equidistant planes are closer together. This is what would be expected if the anisotropic material passed into the crystalline condition in which there would be a closer packing of the molecules, although a change in the distance of the molecular planes might be caused in other ways.

From figure 3 it is evident that $r = D \tan 2\theta$, where D is the distance from the muscle to the photographic plate, r the radius of the diffraction

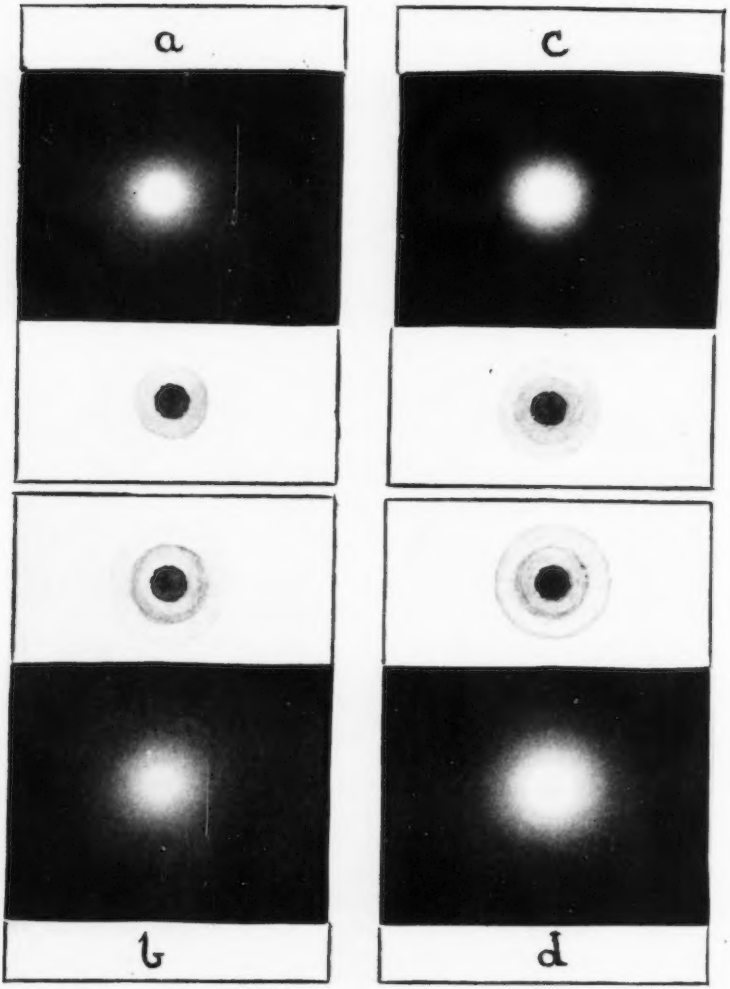


Fig. 4. Diffraction patterns from the sartorius muscle of a frog: (*a* and *c*) relaxed, (*b* and *d*) chloroform or death rigor. The photographs have not been retouched and do not reproduce clearly so that the drawings beneath *a* and *c* and above *b* and *d* have been added to illustrate the differences shown in the originals.

zone or ring, and θ the angle of reflection. By measuring r and D, θ can be calculated. Then d, the distance between the equidistant molecular planes can be calculated from Bragg's formula

$$n \lambda = 2 d \sin \theta$$

In this case λ, the wavelength of the K_α molybdenum rays used, was 0.712 A.U. and n, the order of reflection, was 2 for the inner zone and 3 for the outer zone. The results calculated from figure 4 (*c* and *d*) are given in table 1.

This shows that the distance between the molecular planes, in the anisotropic bands of muscle, is approximately 1 mμ for the relaxed muscle. In the sartorius of the frog the width of a fibril is approximately 1 μ so that the distance between the diffracting molecular planes is about one thou-

TABLE 1

Distance between equidistant molecular planes (d)

ORDER	RELAXED MUSCLE	CONTRACTED MUSCLE
2nd order	9.5 A.U. = 0.95 mμ	8.6 A.U. = 0.86 mμ
3rd order	9.5 A.U. = 0.95 mμ	8.5 A.U. = 0.85 mμ

TABLE 2

Dimensions of molecules

SUBSTANCE	FORMULA	DIAMETER	LENGTH
		A.U.	*A.U.*
Palmitic acid	$C_{15}H_{31}COOH$	4.6	24.0
Stearic acid	$C_{17}H_{35}COOH$	4.7	25.0
Tristearin	$(C_{17}H_{35}O_2)_3C_3H_5$	8.1	25.0
Oleic acid	$C_{17}H_{33}COOH$	6.8	11.2
Triolein	$(C_{17}H_{33}O_2)_3C_3H_5$	11.2	13.0

sandth the width of a fibril. When the muscle is contracted the width of the equidistant planes is 1 A.U. or 0.1 mμ less than in the relaxed condition.

When this work was almost completed a second paper appeared by Herzog (1926), in which he reported an interference fibre pattern from dried muscle indicating a crystal plane distance of 9.94 A.U. Herzog got his best results from muscles dried in a state of tension. I was never able to obtain any diffraction patterns from dried muscles but doubtless they were not dried rapidly enough. The close agreement between my results and those of Herzog, for the distance between the molecular planes, shows that the molecules of the anisotropic band have one of their dimensions equal to 1 mμ (10 A.U.). The molecular dimensions of a number of fats and fatty acids are well known and some of them are given in table 2.

Trillat (1926) and others have found that the reflecting planes in fatty acids are twice the length of the molecule and that the increase in length of a molecule, per carbon atom added, is 1^{+} A.U. Therefore if the anisotropic substance is a fatty acid, cholesterol ($C_{27}H_{46}O$), or lecithin ($C_{41}H_{90}NPO_9$) compound, the length of the molecule would be much too great to give a 10 A.U. spacing. It may be that the 10 A.U. spacing represents the width of a molecule and that under proper conditions reflection from equidistant planes, the length of the molecule, might be obtained also. It would be almost impossible to get the diffraction patterns from molecular planes 40 or 50 A.U. in width with the K-radiation of molybdenum. To get the diffraction pattern from molecular spacings of that width it would be necessary to use a longer monochromatic x-ray, such as the K-radiation from copper.

Discussion. The evidence, as yet, is far from conclusive for or against the theory suggested in this paper, to account for the mechanical shortening in muscular contraction, but tends so far as it goes to support it. While this investigation was in progress a liquid crystal theory of muscle contraction was advanced by Garner (1926) in which he supposes that lactic acid enters into a chemical reaction with the substance on the surface of the anisotropic bands, forming a solid film at the boundary. He gave no proof of his theory and the x-ray evidence so far obtained seems to be more in favor of my hypothesis than that of Garner, because the change in crystal plane distance, that takes place on contraction, argues a change in form throughout the anisotropic segments rather than at the surface only.

Herzog speaks of the fact that he gets a clearer fibre pattern if the muscles are dried in a state of tension. I found that a living muscle in a state of tension gave a diffraction pattern more like that of the contracted muscle, with a well-defined ring at the edge of the zones and a molecular spacing of 8.5 A.U. Herzog thought the material in the anisotropic bands similar to rubber, which becomes crystalline on stretching. It is known that a muscle in a state of elastic tension contracts more promptly and more effectively, for a given stimulus, than one which is relaxed, and smooth muscle can be caused to enter into contraction by stretching. So it may be that a tension causes changes in muscles similar to the changes produced by stimuli to contraction.

The theory of mechanical shortening given here offers a very reasonable explanation of the so-called "catch mechanism" which has been used to explain the fact that muscles, such as the adductor muscles of bivalves, can maintain a contraction with no appreciable consumption of energy. If contraction is due to the formation of the solid crystal modification of the anisotropic liquids in muscles, then, unless a condition occurs that brings about a decrystallization, the muscle would stay contracted indefinitely with no expenditure of energy. In skeletal muscle, neutralization of the

lactic acid occurs as soon as the stimulus is removed and brings about a prompt decrystallization. The permanent tonic state of smooth muscle can be explained by assuming that the state of crystallization, into which the excitation puts the muscle, remains until an inhibitory impulse sets going the opposite process associated with relaxation. Pavlov (1885) has shown that the adductor muscles of bivalve molluscs are supplied with two sets of nerves, exciting and inhibiting. We might assume that excitation of the exciting nerves brings about a state of crystallization and that excitation of the inhibiting nerves is necessary to set going the processes associated with relaxation, and change the solid crystals back to liquid crystal form.

If this view of muscle contraction should ever be accepted it would modify the heat equations of the various processes concerned in muscular contraction. Heat is given out when liquid crystals change into true crystals and the change from a crystal to an anisotropic liquid is accompanied by a definite absorption of heat. Meyerhof (1922) finds that the total anaerobic heat in muscular contraction is 370 calories per gram of lactic acid formed. Hartree and Hill (1923) find that part of this, 296 calories per gram of lactic acid formed, is initial heat and the rest, 74 calories per gram of lactic acid formed, is delayed heat. If Slater's value for the heat of combustion of glycogen (cited from Hill, 1926, p. 61) is taken, the total energy in the formation of one gram of lactic acid from glycogen is $3836-3601=235$ calories. The difference between this value and the initial heat production is $296-235=61$ calories and may reasonably be attributed to the heat of crystallization. In the process of relaxation, the heat of neutralization of one gram of lactic acid, which is 135 calories, minus 61 calories, the heat absorbed on changing from solid crystals back to an anisotropic liquid, gives 74 calories, which is the value found for the delayed anaerobic heat.

SUMMARY

A theory is proposed to account for the mechanical shortening in muscular contraction, in which the hypothesis is made that the substance in the anisotropic bands passes abruptly from the liquid crystal to the solid crystal form, as a result of the increase in acidity due to lactic acid formation.

Assuming that the muscle in a state of contraction consists of crystal layers (anisotropic bands) separated by amorphous layers of thickness d, area A, and surface tension T (isotropic bands), the force of cohesion between the crystal layers is calculated, by the formula that this force equals $\frac{2AT}{d}$ dynes, and found to be about 35 or 40 dynes per muscle fibre, which is not far from the observed experimental value of 55 dynes.

Observations made on the myelin forms of ammonium oleate, under

high power, with polarized light, show that they retract, with the formation of acicular crystals, as the result of a slight increase in hydrogen ion concentration. These observations made the hypothesis a plausible one but gave no direct evidence in respect to its truth.

Direct experimental evidence, concerning the nature and extent of crystallization in muscle fibres, was obtained by means of x-ray diffraction patterns, taken by the monochromatic pinhole method. These photographs showed a series of zones or hazy rings, representing the first, second and third order reflections from equidistant molecular planes. These zones showed two distinct differences between the contracted and the relaxed condition. The first difference was the appearance of a fairly well-defined ring at the edge of the zones, when the muscle was contracted, suggesting an approach to a true microcrystalline condition. The second difference was an increase in the diameter of the zones. This means a smaller distance between the equidistant molecular planes, in a contracted muscle, which would be expected if there were a change from a liquid crystal to a solid crystal modification. The distance between the molecular planes was found to be 9.5 A.U. in the relaxed, and 8.5 A.U. in the contracted state. As this is too short a distance for the length of the long organic molecules involved, it is probable that this distance gives the width of the molecules forming the equidistant planes.

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STUDIES ON METABOLISM

VI. Experimental Hyperthyroidism[1]

MARGARETE META KUNDE

From the Hull Physiological Laboratory of the University of Chicago

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The work of A. Magnus Levy (1904) showed that in man a definite increase in the metabolism occurs after the ingestion of certain thyroid preparations. Following these fundamental observations much work has been done on lower animals, directed towards determining the effects on various physiological processes of increasing or decreasing the amount of thyroid substances present. The literature on this phase has been carefully reviewed by several authors (Carlson, 1912; Kendall, 1915, 1917, 1918, 1919; DuBois, 1916; Grafe, 1923; Zunz, 1924; Dodds and Dickens, 1925). Mention here is made only of those publications having direct bearing on the present research. Carlson (1912) in referring to many of the typical examples of hyperthyroidism points out that the results obtained in many instances are characterized by a lack of uniformity and contradictory conclusions. These discrepancies may be due, in part, to insufficient control of some of the experimental conditions. For example, in this laboratory we have shown repeatedly that the pulse rate of normal dogs, trained to lie quietly, varies from 52 to 72 beats per minute, with an average of approximately 64, and that dogs under normal conditions have an irritable pulse which on the least excitement or muscular activity is increased from 10 to 15 beats per minute, counting the pulse during slight excitement may explain the high rate reported for some normal dogs and the subsequent failure to detect an increased pulse rate during the hyperthyroidism. Again, the potency of the thyroid substance may vary in different samples. But the chief difficulty probably lies in the fact that no basal metabolism tests were made in connection with the animal experiments, hence the investigators were obliged to interpret their findings without the most specific criterion of hyperthyroidism available at the present time. In this report the increased basal metabolism is considered the most important index of the degree of hyperthyroidism experimentally induced.

[1] This work has, in part, been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

EXPERIMENTAL PROCEDURE AND PRESENTATION OF DATA. Seven dogs, chosen from the well-trained animals of the metabolism series, are used in this research. Six of these are normal unoperated animals and one was completely thyroidectomized about two years previous to the observations of this report. The method of determining the basal metabolism (Kunde, 1923) and the care of the dogs in the metabolism series has been previously described (Kunde and Steinhaus, 1926).

The data presented show the influence on the basal metabolism, pulse rate, body temperature and body weight of the ingestion of substances mentioned in the following outline:

I. THYROXIN[2] (Kendall's so-called pure crystalline). A. *Intravenous injections of a single dose.* During 1923 dogs I, II and III each received a single dose of $3\frac{1}{3}$ mgm. on March 15, and on March 26 each received a dose of 10 mgm. On April 9, dogs I and II each received a single dose of 20 mgm. Dog X (1926) received a single dose of 4 mgm. on July 4 and again on August 8. On January 21, 1927, this same dog received a dose of 5 mgm.

B. *Intravenous injections of daily repeated doses.* Dog I (1923) received daily doses of 2 mgm. per dose from December 3 to December 6, inclusive, and 1.2 mgm. per dose daily from December 12 to December 22. Dog V (1923) received daily doses of 2 mgm. per dose from November 6 to November 24, inclusive, and from November 24, 1924 to January 1, 1925, it received daily doses of 2 mgm. Dog X (1925) received daily doses of 4 mgm. from August 25 to August 29, inclusive.

C. *Oral administration.* Dog V. (1925) received daily doses of 4 mgm. to 15 mgm. from February 14 to May 5 inclusive.

II. DESICCATED THYROIDS.[3] Oral administration. A. *Single doses.* During 1923 dogs I and II each received a single dose of 10 grams of desiccated thyroids. On June 18 and July 2 each received a dose of 25 grams and on August 1, September 24 and October 2 each received a dose of 50 grams. In addition to the above, dog II (1923) received a massive dose, of 150 grams, on each of the following days—August 13, September 17 and October 2. On December 19 it received 4 grams. Dog VII received 2.5 grams on April 7, 1926. Dog X received 4 grams August 8.

B. *Daily repeated doses.* Dog II (1923) received 4 grams daily from November 18 to December 22. Dog I received doses of 4 grams to 16 grams from November 24, 1924, to March 22, 1925. Dog V (1925) received daily doses of 2.5 grams from October 7 to November 19. In 1926 it received daily doses of the same amounts from February 22 to

[2] Prepared by E. R. Squibbs & Sons under license of Univ. of Minn.

[3] Prepared by Armour & Co. and containing 0.2 per cent iodine in thyroid combination.

April 18, and again from April 30 to June 3. Dog IV (1926) received daily doses of 2.5 grams per dose from May 4 to June 3, and again from June 20 to July 5. Dog X (1927) received daily doses of 5 grams from February 2 to June 20.

III. TRYPTOPHAN. Intravenous injections. Dog I (1923) received daily doses of 0.1 gram per day from May 15 to May 27 inclusive.

IV. IODINE. A. *Intravenous injections.* Dog V (1923) received daily doses of potassium iodine in amounts varying from 1.2 mgm. to 25 mgm. per kilo body weight from December 8 to December 23 inclusive. During this time the dog received no other medication.

B. *Oral administration* in the form of Lugol's solution given daily during periods of hyperthyroidism experimentally induced. Dog V, 1924, received daily amounts of 15 to 60 drops three times daily from December 24 to December 29 inclusive, and again in 1926 from April 30 to June 3 inclusive. Dog IV, 1926, received doses of 15 to 60 drops three times daily from June 20 to July 5. Dog VII received 15 to 30 drops three times daily from June 20 to July 5, and dog X (1927) received dosages of 15 to 30 drops three times daily.

V. CALCIUM LACTATE, administered orally. A. *To a normal animal.* Dog V (1923) received daily doses of 1 gram per kilo from June 18 to August 11.

B. *To a thyro-parathyroidectomized dog to control parathyroid tetany.* Dog X received 1 gram to $1\frac{1}{3}$ gram per kilo body weight daily excepting during periods of marked hyperthyroidism.

This work has been in progress more than four years (preliminary reports, Kunde, 1924.) During this time, in many instances, daily metabolism tests were made on these dogs over many months. Space will not permit a detailed publication of all of the results but a compilation of the data, showing the most significant changes induced, by each experiment or series of experiments, is contained in tables 1 and 2. (The term "experiment" as here used indicated a determination of results on the basal metabolism, body temperature, body weight and pulse rate of the ingestion of a single dose, or a series of daily doses of any substance mentioned in the outline.) Tables 1 and 2 give a summary of the above results in terms of per cent changes in the basal metabolism per square meter of body surface. Also the per cent loss in body weight and the number of days required for the metabolism to return to normal after discontinuing the experiment and remarks showing when Lugol's solution was used. Table 3 contains daily data on experiments with dog I, showing mild to fatal hyperthyroidism induced in a normal dog by feeding desiccated thyroid in doses of 4 to 16 grams daily.

A graphic presentation of the effect on the basal metabolism, body weight and pulse rate induced by daily injections of thyroxin with and without

Lugol's solution; of intravenous injection of potassium iodide and of feeding desiccated thyroids with and without Lugol's solution is given in figure 1 (*A*, *B*, *C*, *D*, *E*.)

TABLE 1

Summary of the results obtained by administering single doses of Kendall's thyroxin (intravenously) or desiccated thyroids (orally) to dogs

The effects on the basal metabolism in tables 1 and 2 are tabulated in terms of per cent deviation from the normal. All calculations in these tables are based on the total number of calories of heat produced per square meter of body surface per 24 hours. Changes of 2 to 4 per cent fall within the limits of normal variations and are tabulated here merely to indicate no influence of the thyroid substance. A change of 0.3 kilo or more in body weight is considered sufficiently great to call forth a new determination of the surface area. Seven dogs were used in these experiments. The third column shows the per cent change in the metabolism 7 to 12 hours after giving the dose, while the fourth column shows the maximal effect, which, for these doses, always occurred on the second day. The fifth column shows the number of days required for the metabolism to return to normal after a single dose.

NUMBER OF TESTS	QUANTITY OF EACH DOSE	EFFECT ON BASAL METABOLISM 7 TO 12 HOURS AFTER DOSE	MAXIMUM EFFECT ON BASAL METABOLISM SECOND DAY	DAY OF RETURN TO NORMAL
		Thyroxin		
3	3⅓ mgm.	−2 to +3	+5 to +10	2
2	4 mgm.	Not determined	+18 to +36	2 to 6
1	5 mgm.	Not determined	+5 to +6	8
3	10 mgm.	0 to +5	+6 to +22	3 to 6
2	20 mgm.	0 to +3	+6 to +13	3 to 4
		Desiccated thyroid		
1	2½ grams	+1	+2	
2	4 grams	−3 to +1	0 to +1	
2	10 grams	0 to +2	+9 to +18	3 to 5
2	25 grams	Not determined	+12 to +14	3 to 6
6	50 grams	*	+5 to +24	3 to 5
3	150 grams	*	+32 to +43	3 to 8

* Not determined because the specific dynamic effects of the protein would obscure the thyroid effects.

Discussion of results. *The basal metabolism and body temperature as influenced by* 1, *single doses of thyroxin injected intravenously.* The evidence summarized in table 1 clearly indicates that we cannot predict what the metabolic response to a single dose of thyroxin, varying in amount from 3⅓ to 20 mgm. will be. From 7 to 12 hours after injecting these amounts there may be no change in the basal metabolism or there may be

a slight depression of approximately 2 per cent, or there may be an increase of 5 per cent over the level at the time of the injection, but these results usually fall within the limits, or approximate, the extremes of the normal variations. That the changes bear no quantitative relationship to the amount of thyroxin injected can be clearly seen by examining the protocols of dogs I, II and III. The response induced by injecting $3\frac{1}{2}$ mgm.

TABLE 2

Summary of results obtained by the administration of daily doses of Kendall's thyroxin or desiccated thyroids, with and without Lugol's solution, to dogs

NUMBER OF DOG	DOSE	DURATION OF EXPERIMENT	MAXIMUM EFFECT ON B.M.R. (DAY OF EXPERIMENT)	RETURN TO NORMAL OF B.M.R.	MAXIMAL LOSS IN BODY WEIGHT	REMARKS
	A. Daily doses of Kendall's thyroxin (intravenously)					
		days		*days*		
I	1.2 mgm.	13	48% (14th)	8	6%	No Lugol's
I	2.0 mgm.	4	17.4 (3rd)	3	2%	No Lugol's
V	2.5 mgm.	19	73% (20th)	7	2%	No Lugol's
V	2.5 mgm.	39	121% (29th)	16	7%	Lugol's 31st to 35th day
X	4.0 mgm.	5	72% (6th)	7	8%	No Lugol's
	B. Daily doses of thyroxin (orally)					
V	4–15 mgm.	81	119% (70th)	11	19%	No Lugol's
	C. Daily doses of desiccated thyroid					
IV	$2\frac{1}{2}$ grams	31	61% (31st)	15	1%	No Lugol's
IV	$2\frac{1}{2}$ grams	16	52% (16th)	4	5%	Lugol's entire time
V	$2\frac{1}{2}$ grams	56	104% (56th)	9	11%	Lugol's last 29 days
V	$2\frac{1}{2}$ grams	44	120% (45th)	5	5%	No Lugol's
V	$2\frac{1}{2}$ grams	35	88% (33rd)	7	2%	Lugol's entire time
VII	$2\frac{1}{2}$ grams	35	78% (35th)	13	4%	No Lugol's
VII	$2\frac{1}{2}$ grams	16	66% (16th)	5	8%	Lugol's entire time
II	4 grams	34	25% (29th)	10	6%	No Lugol's
I	4 grams to 16 grams	119	110% (92nd)		35%	Died of hyperthyroidism 120th day
X	5 grams	138	170% (67th)			Lugol's 40th to 67th day

of thyroxin intravenously into each of these dogs at the end of 7 to 12 hours ranges from −2 to +3 per cent. On April 9th, dogs I and II each received a dose of 20 mgm. 7 to 10 hours after these injections there was no effect on the metabolism of one dog and an increase of only 3 per cent in the other. But closely following this there is a distinct increase in the heat production which usually reaches a maximal on the 2nd day and never later than the 3rd day. This maximal increase in the heat production due

to a single dose of thyroxin is no more proportional to the amount of thyroxin injected than the increase apparent at the end of 7 to 12 hours. For example, on March 28, 1923, the heat production of dog II showed a maxi-

TABLE 3

Data taken from the protocols of dog I ♀, (with thyroid glands intact) showing the basal metabolism during mild to fatal hyperthyroidism induced by feeding 4 grams to 16 grams of desiccated thyroid daily for 119 days

DATE (1924-1925)	TOTAL CALORIES PER 24 HOURS	PULSE PER MINUTE	BODY TEMPERATURE (RECTAL)	BODY WEIGHT
			°F.	kilos
November 4 to 23	422*	63	100.2	10.1
November 24	420	64	100.4	10.1
4 grams thyroid daily†				
November 25	463	62	101.2	10.1
November 26	496	92	101.2	10.1
November 27	557	96	101.2	10.1
November 28 to December 4	545	95	101.4	10.1
8 grams thyroid daily				
December 5–14	637	118	101.2	10.1
December 15–26	636	116	101.7	9.9
December 27 to January 2	656	76	100.7	9.6
January 2–19	653	91	100.6	9.5
January 20–24	598	100	101.4	9.4
January 25 to February 1	573	94	100.4	9.1
February 2–9	572	95	101.2	9.1
February 10–16	583	91	101.8	8.9
16 grams thyroid daily				
February 17–26	666	96	101.7	8.6
February 27 to March 7	648	96	101.8	8.0
March 8–18	673	134	102.3	7.6
March 19	640	120	102.0	7.5
March 20	740	120	103.0	7.3
March 21	742	122	103.0	7.1
March 22		188	108.4	6.6
Dog died of hyperthyroidism				

* Average of 10 normal tests.

† Average results of daily tests over time indicated.

mal increase of 24 per cent as a result of the intravenous injection of 10 mgm. of thyroxin two days previously. On April 9 this same dog received 20 mgm. with a maximal increase of only 10 per cent in the metabo-

lism. This increase in heat production is always accompanied by a rise in body temperature. Usually the most marked metabolic reaction is accompanied by the greatest rise in body temperature. The exceptions to this are probably due to discrepancies which occur between the rectal temperature and the general body temperature. Barr et al (1922) showed that the rectal temperature indicates only in a general way changes in the average body temperature, and that it is possible to have a rise in the rectal temperature while there is a fall in the average body temperature or *vice versa*. The increased temperature, *per se*, on the basis of the relationship between fever and heat production is sufficient to cause a goodly portion, but not all of the increased metabolism. A portion must still be attributed to the specific influence of the thyroxin.

In all cases, after single intravenous doses of thyroxin, in total amounts up to 20 mgm. per 10 kilo dog, the basal metabolism and body temperature both return to normal in 3 to 5 days after the injection. Following this there may be a day or two of subnormal metabolism after which the heat production turns to and remains normal. This rapid disappearance of the metabolic stimulus was observed in the myxedematous dog as well as in the normal dogs. But in the myxedematous dog the increased metabolism was higher for 1 to 3 days after the injection and the maximal effect usually occurred on the 3rd day instead of the 2nd. In this respect our results markedly differ from the observations of clinical cases reported by Plummer and Boothby (1921). These authors have reported that the greatest metabolic reaction of a single intravenous injection of thyroxin most frequently occurs on the 8th day, and later Boothby and Sandiford (1924) that the effect may still be markedly apparent 48 days after the thyroxin was injected.

2. *Single doses of desiccated thyroid*, administered orally in amounts varying from $2\frac{1}{2}$ grams to 50 grams per 10 kilo dogs, like thyroxin, have little or no effect on the basal metabolism for 7 to 12 hours. But from then on there is a marked increase in the heat production lasting 2 to 4 days with an increase in body temperature of 0.2 to 0.4°F. This is less fever than occurs with thyroxin. Here again there is no evidence of a quantitative relationship between the amount of desiccated thyroids fed and the increase in the basal metabolism (table 1). On June 18, 1923, dog I received 10 grams desiccated thyroids, resulting in a maximal heat production of 9 per cent above normal, which occurred two days after feeding the thyroids. On July 2 this same dog received 25 grams with a maximal rise in the basal metabolism of 14 per cent. On August 1 it received 50 grams with a maximal response of only 5 per cent. After a massive dose of 150 grams the heat production may increase to 43 per cent above normal. This may occur at the end of the first day of ingestion with an increase in body temperature 2°F. or less. This rise in body temperature is compar-

able to that which occurs after moderate doses of thyroxin, but the increase in metabolism is approximately 100 per cent greater. Three to 8 days after giving a massive dose of thyroid the basal metabolism returns to normal, after which there may be a slight depression lasting only a few days with no further effect of the thyroid feeding.

In comparing the results of a single moderate dose of thyroxin or desiccated thyroids, we fail to find any advantage of the intravenous method of introducing the thyroid substance. In fact, distinct disadvantages are apparent. The evidence at hand gives no support to the argument in favor of the intravenous method because of more rapid effects, for in either case (oral or intravenous routes) 7 to 12 hours must elapse after giving the thyroid substance before a measurable increase in the heat production occurs. The disadvantages of the intravenous routes which are most apparent are, *1*, the greater rise of body temperature after moderate doses accompanying a less increase in heat production, which suggest the presence of minute quantities of toxic substances causing fever. It is quite possible that after massive doses of desiccated thyroids (150 grams) sufficient amounts of the same substances are absorbed to be responsible for some of the observed rise in body temperature. *2*, Biological evidence of a foreign substance present in varying amounts in different samples of thyroxin have been repeatedly demonstrated, by degenerative changes which occur in the aorta of cretin rabbits (Carlson and Kunde) after intravenous injections of minute quantities of thyroxin. This does not occur after oral administration of desiccated thyroids. The nature of this toxin is entirely unknown. According to Kendall, thyroxin is a pure crystalline compound. Chemical tests have evidently failed to reveal any impurities in the substance, but it is well established that highly toxic substances of organic nature may be present in such minute quantities as to escape detection by chemical tests and yet cause most severe biological reactions (Wells, 1920).

3. *Daily doses of thyroxin injected intravenously with and without the administration of Lugol's solution.* Table 2 contains the summary of 5 experiments in which thyroxin was given daily.

The results of 2 experiments with dog V extending over 19 and 39 days respectively are graphically shown in figure 1 (A and C). In each of these, the dog received the same amount of thyroxin daily, namely, 2.5 mgm. The striking features of both series is the gradual increase in the metabolism which occurs as the time progresses. On the 5th day of the first series the metabolism was 38 per cent above normal. On the 17th day it had increased to above 65 per cent and on the 19th day attained a height of 73 per cent above normal. The thyroxin was discontinued at that time because the condition of the dog closely resembled the clinical state of crisis described in some severe cases of toxic goitre. The body temperature

was 6°F. higher than on the initial day of the experiment; the pulse rate 168 per minute as compared to a normal of 62, and an increase of 3 to 4 fold on the respiratory rate. In addition to this, peculiar skin lesions of a severe type appeared, which will be more fully described in the following paragraphs. In the 2nd experiment, the progressive increase in metabolism is

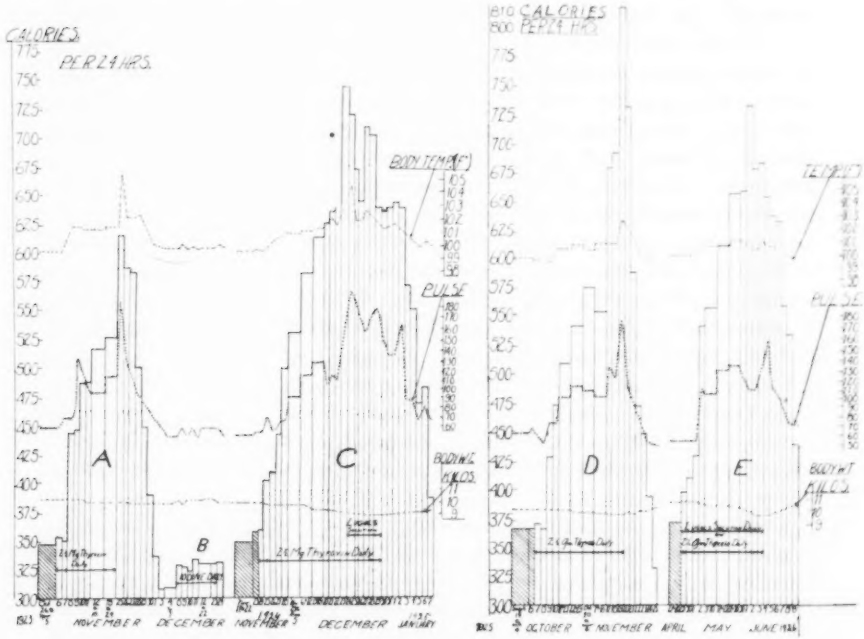

Fig. 1. Graphic presentation of the basal metabolism, pulse rate, body temperature and body weight of dog V, during ingestion of daily doses of Kendall's thyroxin or desiccated thyroid with and without Lugol's solution; and during a period of intravenous ingestion of potassium iodide. The broad vertical bars = averages of 5 or more metabolism tests made on consecutive days. The narrow bars = single determinations. The cross hatchings = controls. *A* = 19 daily doses of thyroxin (intravenously). *B* = 16 daily intravenous injections of potassium iodide (1.2 mgm. to 25 mgm. per kilo of body weight). *C* = daily thyroxin and Lugol's solution during the crisis. *D* = feeding 2½ grams desiccated thyroids daily. *E* = 2½ grams desiccated thyroids daily and Lugol's solution during this entire experiment.

even more apparent. The range of increase in metabolism extended from 44 per cent on the 5th day of the series to 71 per cent on the 17th day and the occurrence of crisis with a metabolic rate 121 per cent above normal on the 29th day. This crisis was ushered in by the same marked increase in

body temperature, pulse rate and respiratory rate as was described above, but at this time in spite of the critical condition of the dog the administration of thyroxin was continued for 9 more days with 15 to 30 drops of Lugol's solution daily for 6 days. There was a drop of 10 per cent in the metabolism on the day after the appearance of the crisis (30th day of series) before the iodine was given. On the 33rd day the metabolic rate had decreased only 3 per cent—a negligible fluctuation. On the 35th day it had dropped to 77 per cent above normal. The Lugol's solution was now discontinued and the thyroxin given for 3 more days with no change in the condition of the dog. No amelioration of symptoms during this crisis seems attributable to the iodine therapy. There was a decrease of 44 per cent in the metabolism but 10 per cent of this occurred before the iodine was given. The loss in body weight, tachycardia, fever and rapid irregular pulse with a basal metabolism rate of more than 77 per cent above normal persisted through the entire crisis. The withdrawal of the iodine the last three days of the experiment did not aggravate the symptoms. More evidence of the negative influence of Lugol's solution in experimentally induced hyperthyroidism is given under the discussion of the influence of thyroid feeding.

4. *Daily doses of thyroxin administered orally* in quantities of 4 to 15 mgm. per 10 kilo dog for 81 days cause approximately the same response in the basal metabolism as has been described after intravenous injection with these exceptions: The crisis may occur several weeks later with a maximal temperature of 103° instead of 105°F.

5. *Daily doses of desiccated thyroids with and without Lugol's solution.* The summary of 10 experiments with 6 dogs to which desiccated thyroids were fed daily over a long period of time is contained in table 2. Figure 1, *D*, graphically presents the results of an experiment 44 days in duration (October 7 to November 19, 1925) during which $2\frac{1}{2}$ grams of thyroid were fed to dog V daily, without Lugol's solution. The progressive increase in the metabolism, resulting from the daily ingestion of a constant dose, is as follows: On the 5th day of the experiment the metabolism was 33 per cent above normal; on the 17th day it was 50 per cent above normal, and on the 45th day crisis occurred with a metabolic rate of 120 per cent above normal, body temperature 103°F. Thyroid feeding was discontinued and within 7 days the metabolism had returned to its normal level. Later (February 22 to April 18, 1926) this experiment was repeated, with these exceptions: the administration of desiccated thyroid extended over 56 days and beginning on the 28th day and continuing throughout the remainder of the experiment (28 days), 15 drops of Lugol's solution were given 3 times daily. No improvements in the toxic symptoms greater in magnitude than fluctuations, which occur without the iodine, are in evidence. Twenty-nine days after beginning the iodine therapy and the 57th

day of the experiment the basal metabolism was 104 per cent above normal with a body temperature of 103°F. This is almost identical to the crisis which occurred without the iodine. In an attempt to determine whether the toxic symptoms could be held in abeyance, or a crisis prevented by early iodine treatment, another experiment extending over 35 days (April 30 to June 3, 1926) was performed, during which iodine in doses mentioned above was given throughout the entire period of thyroid feeding. In terms of per cent above normal, the following figures were obtained: on the 17th day of the experiment 50 per cent; on the 29th day 75 per cent; and on the 35th day 65 per cent. Similar experiments to this, with dogs IV, VII and X (table 2) show that massive doses of Lugol's solution not only fail to prevent or reduce the toxic symptoms caused by the ingestion of thyroid substances but may slightly aggravate the condition.

This ineffectiveness of iodine therapy in experimental hyperthyroidism is very different from results reported in clinical cases. It is now well established that temporarily, at least, the symptoms of toxicity in many patients with toxic goitre are reduced with Lugol's solution (Kocher, 1895; Plummer and Boothby, 1924). Due consideration, however, must be given to the important difference of hyperthyroidism experimentally induced from the hyperthyroidism as it appears clinically. In the first instance the preformed thyroid substance is introduced into the system, either directly by way of the blood steeam, or by way of the alimentary canal. Here the iodine is wholly ineffective. Clinically, hyperthyroidism is associated with a disturbed condition of the thyroid gland. In such cases, in some instances, temporarily at least, the symptoms of toxicity are markedly reduced after iodine therapy. This together with known facts relative to the effect of iodine on the thyroid gland (Kocher, 1895; Marine, 1907, 1914, 1921; Bensley, 1914) suggests that the mechanism by which it exerts its influence is chiefly through this gland.

The experiment on thyroid administration to dog I, terminated fatally after 119 days (November 24, 1924 to March 22, 1925) of feeding 4 to 16 grams daily.

Death due to experimental hyperthyroidism. The same gradual increase in the metabolism as time progressed, was observed in dog I as has been described, although the initial doses of 4 grams daily (nearly twice the amount given dog V) was doubled from time to time with the following results. From November 24, 1924 to January 2, 1925, it received 4 grams daily. On the 5th day of the experiment the metabolism was 30 per cent above normal. On the 17th day it was 50 per cent above normal, and on the 40th day it was 58 per cent above normal. From January 2 to February 9, the dog received 8 grams daily. On February 9 (77th day of the experiment) the metabolism was only 40 per cent above normal. A decline in the metabolism amounting at times to 18 per cent was apparent

from January 23 to February 16 despite the fact that during this period 2 to 4 times the quantity of desiccated thyroids were ingested as where the metabolism held at + 58 per cent. From February 9 to March 22 the dog received 16 grams daily. The highest metabolism occurred February 23 (93rd day of the experiment), when it reached 110 per cent above normal with a body temperature of 102°F. Following this there was a temporary drop of 26 per cent in the metabolism. From March 1, 1925, to March 22 it varied from 84 to 92 per cent above normal. These peculiar drops of 26 per cent or more are always accompanied by a diminution in body temperature and a decrease in the pulse rate, which closely resembles the remissions reported in toxic goitre patients Clinically, we may assume that the remission is due to a temporary decrease in the output of an over-active gland, so that the actual amount of thyroid substance poured into the blood stream is lessened. This is probably not the true explanation, because during the remission in experimental hyperthyroidism the amount of thyroid substance was never diminished but in some instances actually increased twofold, a few days prior to the occurrence of a decline in the metabolism. On March 21 the dog seemed somewhat more active than usual, it was exceedingly alert and easily excited. It greedily ate food and drank much water. The body temperature had been 103°F. for two days. The pulse rate was not markedly increased over what it had been and the basal metabolism was 94 per cent above normal. On the following day at the usual hour of making the metabolism tests the dog was found lying in the cage unconscious and in a moribund state. It had lost 0.5 kilo in body weight during the past 24 hours. Enophthalmos was present. The number af respirations was 168 per minute, with tongue protruding. The body temperature was 108.4°F. (an increase of 8.4° above normal). The pulse rate was 188 per minute as against a normal rate of 62. This condition lasted about one hour when respiration ceased several minutes before the pulse stopped beating. Death seemed essentially due to hyperpyrexia and a disturbance of the cardiac rhythm.

Autopsy findings. The heart was firm and large and continued to fibrillate several minutes after the thoracic cavity was opened. The clotting time of the blood was much delayed. The thyroid appeared markedly atrophied, both lobes weighing only 580 mgm. The pancreas was mottled with tiny flecks on the surface. The liver seemed large, dark red and cut with gritty feel. The spleen was bluish brown and weighed 1.845 gram. The hypophysis was hemorrhagic and weighed 55 mgm. The bladder was full and distended. The alimentary tract seemed essentially negative excepting for a hemorrhagic patch about 1 inch in diameter near the pylorus; the rectum contained a small amount of blood-stained mucus and some pasty fecal material.

Gastro-intestinal symptoms in experimental hyperthyroidism. During

the initial days of the ingestion of the thyroid substance, a loss of appetite which in some dogs may develop into anoxemia is apparent. But beginning at about the 7th to the 10th day of the experiment there is a noticeable increase in the appetite which soon becomes so intense that the dogs greedily devour their daily ration. This increased desire for food continues up to the very day of death. No vomiting was ever observed. The stools at this time are neither frequent nor fluid but consist of soft unformed fecal masses more offensive than the normal dog's stools. Defecation is accompanied by much tenesmus. After the fecal mass is passed the dogs may circle about the booth in the defecation posture with ineffective and apparently painful straining. This may result in the expulsion of fresh blood and frothy gelatinous mucus. The diarrhea can always be controlled by adding bones to the dietary. Later the stools become frequent and more fluid in character closely resembling those described in fermentative diarrhea.

The effect on the body weight of dogs varies within wide limits. All dogs received a dietary which consisted of maintenance rations before the beginning of the experiment. In spite of this, dog IV lost less than 1 per cent of the initial weight during 31 days of hyperthyroidism. This peculiar ability of some dogs to maintain their normal weight under these toxic conditions was observed by Carlson et al (1912). Dog I lost 35 per cent of its initial weight during a period of 119 days of thyroid feeding which terminated fatally (table 2). The most rapid loss in body weight occurred in the myxedematous dog although this animal received much less thyroid per kilo of bodyweight than the unthyroidectomized animals. Our results indicate that neither the amount of thyroids fed nor the height of the metabolic rate determines the per cent loss in body weight. But some condition perhaps dependent upon the nutritional state of the animal or an undetermined selective resistance to a toxemia which is not measured in terms of increased metabolism is the determining factor.

Cardiac and nervous symptoms. The first perceptible result on the heart is a distinct increase in the force of the beat, apparent about 10 hours after the ingestion of thyroid substances. This is followed by a definite increase in irritability such that under ideal conditions of rest there may be no increase in the pulse rate, but the slightest movement or excitement accelerates the rate 15 to 20 beats per minute. Following this and usually apparent the 1st and 2nd weeks of the experiment, the rate may be accelerated from approximately 60 to 88 or 90 beats per minute. At the time of death it may reach 188 per minute (table 3). By the 2nd or 3rd week a disturbance of rhythm can be detected by a complete abolition of the sinus arrhythmia, which in the dog is a normal phenomenon. The complete disappearance of this irregularity suggests a change in the control of the sino-

auricular node in which the vagus influence is important. Later a distinct loss of a beat can be detected, and in the dog that came to autopsy as a result of hyperthyroidism, the heart continued to fibrillate vigorously several minutes after respiration ceased.

The first definite influence on the nervous system observed in this study seems to be of vagus origin and consists in complete obliteration of the sinus arrhythmia. Following this, after several weeks, an increased restlessness and hyperexcitability is easily apparent but no objective way of measuring this suggests itself at present.

Skin lesions. All dogs used in experiments extending over several weeks or months have at various times displayed transient patches of erythemia, which, judging from the behavior of the animals, give rise to intense itching and become slightly injected. In dog V a most acute and severe type of skin lesion became apparent with the onset of each crisis. This always occurred as a localized area upon either the head, neck or shoulders, and covered a patch several inches in circumference. It was always ushered in suddenly as an erythematous plague which itched so intensely that the dogs' paws had to be bound to prevent laceration of the site. In a very short time the patch became infiltrated and a huge bulla formed which promptly perforated leaving an angry looking inflammatous, area. This condition was first observed on the 19th day of thyroxin injections. The thyroxin was discontinued and within three days the skin lesion showed marked signs of healing. A similar condition occurred at the onset of crisis the following year, but this time the thyroxin was continued for 8 more days with the addition of Lugol's solution (15 to 60 drops 3 times daily). The skin lesion showed no signs of healing until the thyroxin was discontinued, whereupon healing promptly occurred. In this dog similar skin eruptions have been observed at the onset of crises due to thyroid feeding.

Iodine. The peculiar skin lesions described above closely resembled the clinical condition of dermatitis medica-mentosa due to the ingestion of iodides. In as much as 65 per cent of the thyroxin molecule consists of iodine, it was thought that by intravenously introducing the thyroxin sufficient iodine might be directly introduced into the blood stream to be responsible for the skin eruptions, and directly or indirectly account for a portion of the increased metabolism. Figure 1 *B*, graphically shows that iodine injected intravenously over the same number of days and in amounts equal to twice the iodine content of the dose of thyroxin was ineffective in producing skin lesions. There was also a negligible influence on the basal metabolism. Magnus Levy (1904) showed that in man iodine caused no influence on the basal metabolism rate.

Exophthalmos does not occur as a part of the symptom complex of experimental hyperthyroidism in the dog. This was demonstrated by Carlson

(1912) and confirmed many times in the present research. No protrusion of the eyeballs was observed at any time in these dogs, in fact, during the moribund state marked enophthalmos was apparent. This cannot be interpreted as evidence supporting the theory that experimental hyperthyroidism does not simulate the clinical symptoms of toxic goitre, but must result in some circulatory or anatomical difference in the dog which prevents the eyeball from protruding, inasmuch as both desiccated thyroid and crystalline thyroxin from samples which failed to produce exophthalmos in the dog are effective in producing exophthalmos in the rabbit.

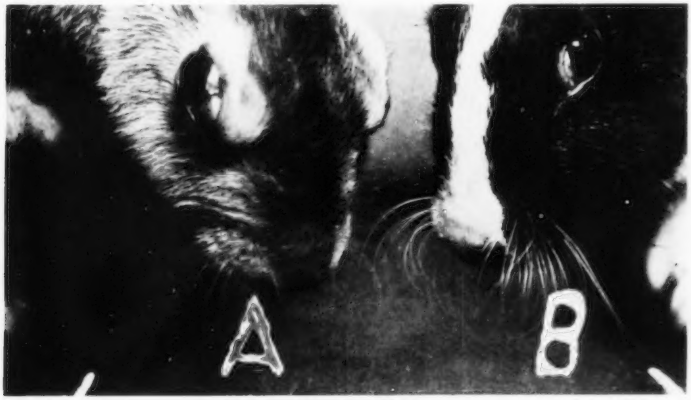

Fig. 2. Showing two adult female rabbits. Each rabbit has received 250 mgm. of desiccated thyroids daily for 6 weeks. Rabbit A shows marked exophthalmos, whereas this condition in rabbit B (normal animal) is present to a very slight degree if at all. Rabbit A was completely thyroidectomized between 2 and 3 weeks after birth, but received small amounts of thyroid substances during the growth period. Subsequently it was allowed to develop myxedema for many months by discontinuing the thyroid administration. A very slight bulging of the eyes becomes apparent at this time but this condition is markedly exaggerated as the previously myxedematous animal becomes toxic with thyroid feeding.

Normal rabbits made toxic with thyroid substances develop a questionable exophthalmos, a wide palpebral fissure and a dilated pupil. If rabbits, completely thyroidectomized when young, and given small doses of thyroid substances so as to stimulate growth, are allowed to develop myxedema by discontinuing the administration of thyroid substance for several months, then fed toxic doses of thyroid for several weeks, marked exophthalmos occurs, which becomes greatly accentuated if the return circulation from the head is embarrassed by holding the rabbit by the skin of the back in such a manner that the back is arched and elevated while the head hangs down (see fig. 2). Tatum (1913) showed that in cretin rabbits there is

much degeneration of the myocardium. This weakened circulatory mechanism during the period of myxedema may result in congestion which according to Charles H. Mayo (1922) favors the accumulation of pads of fat in various depots of the body. The accumulation of fat back of the eyeball may favor the development of exophthalmos, but this is not the only mechanism involved for although slight protrusion of the eyeballs

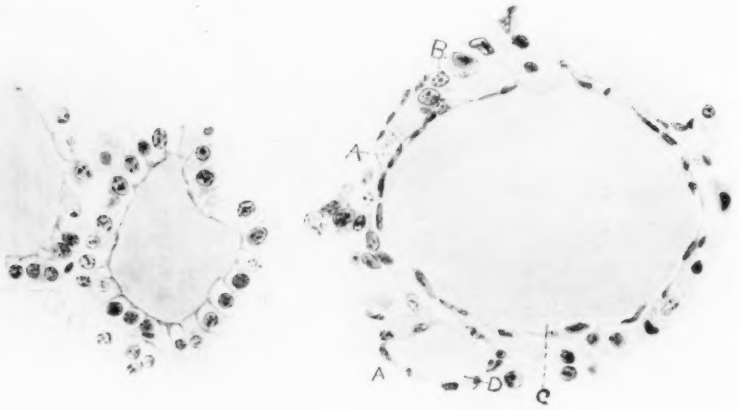

Fig. 3. (N)—Portion of a section through the thyroid gland of a normal dog (dog H). The follicles are lined with closely packed high cuboidal epithelial cells, *E*. The basal metabolism of the dog was 782 calories of heat per sq. m. of body surface per 24 hours. The dog met with an accidental death a few hours after the determination of the heat production.

(H)—Portion of a section of the thyroid gland of dog I after 119 days of experimental hyperthyroidism with fatal termination. The basal metabolism of this dog on the day previous to death was 94 per cent above normal. Here both large and small follicles are lined with *A*, elongated epithelial cells with oval nuclei, fibroblastic in appearance. The normal arrangement of the secreting cells, conforming to an outer and inner pole has given place to a polarity parallel with a line circumscribing the colloidal mass.

B, Retrograde epithelial cells; *C*, large follicle; *D*, small follicles (Camera lucida 8 × 2).

may be observed in the myxedematous condition, *it becomes marked only after toxic symptoms of hyperthyroidism have developed.*

The thyroid gland in late experimental hyperthyroidism. Grossly there is marked atrophy of the gland, the total weight being 580 mgm. per 8.6 kilo dog. Figure 3, *B*, shows a portion of a section of a gland with several follicles of different sizes. These follicles contain colloid and are lined with a scant number of elongated cells, fibroblastic in appearance, which have

completely lost the cuboidal contour of the normally secreting cells. Throughout the gland advanced stages of follicular degeneration and disintegration could be seen. Retrograde epithelial cells were numerous, and the gland seemed completely disorganized.

If desiccated thyroids supply all of the active principle of the normally secreting gland then the above changes may be the result of simple atrophy of disuse, or the large quantity of iodine introduced with the thyroid over a long period of time may result in these changes which may be a terminal state of the involution described by Bensley (1914) after the administration of iodides. Jackson (1925) describes changes occurring in the thyroid gland during inanition and notes that in the follicles, the epithelial cells may show simple atrophy with desquamation of degenerated cells. Wilson (1922) notes that in late exopthalmos many follicles containing colloid are lined with flattened parenchymal cells, but in some instances newly developed cells are numerous.

The morphological changes in the follicular epithelium described in the above citations are limited to a flattening of the epithelial cells. No description of a change resulting in an elongated follicular cell with a polarity parallel to a line circumscribing the colloid instead of the normal outer and inner pole arrangement has been found. Further experiments directed toward determining whether this is due to extreme inanition, excessive iodine administration or a selective condition caused by the ingestion of the thyroids is in progress.

Hyperglycemia and experimental thyroidism. Spontaneous glycosurease and hyperglycemia frequently occur in patients suffering from hyperthyroidism. Denis, Aub and Minot (1917) reported that fasting hyperglycemia was extremely rare, whereas alimentary hyperglycemia occurred in every case studied by these authors. Neither hyperglycemia nor glycosuria could be induced in these dogs by thyroid feeding even when the metabolic rate was increased to + 110 per cent and a loss of 30 per cent in body weight had occurred. During the period of recovery from hyperthyroidism, especially at the time when the basal metabolism is apt to be slightly below normal, a slight fall in the blood sugar seems to be apparent. This is at a time when the weight lost during the thyroid feeding is rapidly recovered. Five analyses of the blood sugar of dog F, during the 7th month of an experiment in which 20 grams of desiccated thyroids were fed daily (body weight 8.2 kilos) show that the range of variation in blood sugar fell within 92 to 96 mgm. per 100 cc. of whole blood. Numerous qualitative tests failed to reveal the presence of sugar in the urine of these dogs even during periods of most marked toxicity.

The mode of the action of the thyroid hormone—Boothby (1924) quoting Plummer, states that "The active agent of the thyroid gland is a catalyst that accelerates the function of a quantum of potential energy in

the cells of the organism." Sandiford (1920) states that "The determination of the metabolic rate gives a very accurate mathematical index of the degree of functional activity of the thyroid gland." Boothby (1924) maintains that "Thyroxin can be considered as a catalytic agent which increases the metabolic rate in proportion to the quantum actively present (the concentration of the cells of the body)." C. H. Mayo (1922) states that "The thyroid has to be with metabolism making possible the exchange of gases in the cell somewhat as the little boy pulls out the draft of the stove and makes the fire burn."

Our results show that in the dog, after administering thyroid substances, either intravenously or per os, no effect on the metabolism occurs for approximately 7 hours. If daily repeated doses are given this delay is followed by a progressive increase in the heat production (with wide fluctuations) as time advances, reaching a maximum three weeks or more after the ingestion of the initial dose. This seems to indicate that either the thyroid substance must be stored in the cells of the body in a simple accumulative manner, or no relationship exists between the amount of thyroid substance present and the height of the metabolic reaction. The evidence resulting from our experiments indicates that a quantitative relationship between the degree of hyperthyroidism produced and the quantity of thyroid substance administered does not exist, but the height of the response to a quantity of thyroid substance depends upon some condition of the body cells, which becomes less and less resistant to repeated doses of a given quantity of the thyroid hormone. This condition seems to be closely related to water metabolism; the toxicity of hyperthyroidism being associated with dehydration phenomena (Kunde, 1926), whereas in myxedema hydration is present. Experiments in progress show that a marked polyuria and polydipsia develop in rats, rabbits and dogs made toxic by the ingestion of thyroid substances. The polydipsia in a dog weighing 8.2 kilos and receiving 20 grams of thyroid daily is such that the water intake over many weeks ranges from 2900 cc. to 7000 cc. per 24 hours. Some dogs develop only a slight polydipsia. Toxic signs occur much more rapidly under these conditions than when large quantities of water are consumed daily.

After several weeks of hyperthyroidism a decrease in metabolism amounting to as much as 18 per cent may occur at a time when twice the amount of thyroid substance is ingested as during a previous period when the metabolism is much higher. This phenomenon is quite comparable to the remission and exacerbations reported in toxic goitre and suggest that the underlying cause of the fluctuations are not due to a simple increase or diminution of the substance elaborated by the thyroid gland, acting as a catalytic agent.

The initial delay is not due to time involved in absorption from the gas-

tro-intestinal tract because the same latent period occurs after intravenous injections of thyroxin. Since it has been demonstrated by the precipitin reaction (Carlson and Hektoen, 1925) that thyreoglobulin has a high degree of specificity, the possibility of a delay due to time consumed by the thyroid gland in converting the substances ingested into a more specific biological product must be considered, but can readily be dismissed because completely thyroidectomized animals manifest the same initial latent period and later both thyroidectomized dog and rabbit develop toxic symptoms faster and in a more marked degree than the normal animal receiving the same quantities of thyroid substance daily, indicating that *some peculiar condition of the tissue determines what the response to a given amount of thyroid substance will be.*

The basal metabolism of dog V was studied after the intravenous administration of tryptophan because of the similarity between the structural formula of tryptophan and the formula for thyroxin proposed by Kendall (1919) and generally accepted at the time these observations were made (1923). After 13 daily doses of 0.1 gram of tryptophan no increase in the metabolism occurred, in fact, a decrease is apparent, but by reason of the limited number of observations no significance should be attributed to this other than a failure to produce stimulating effects. Recent reports of Harington (1927) indicate that the structural formula of thyroxin does not resemble tryptophan.

Calcium lactate administered orally in doses of 1 gram per kilo of body weight per day has no influence on the heat production of normal dogs. This observation was made to assist in interpreting the results obtained from studies of the basal metabolism of dog X, which had been thyroparathyroidectomized by Dr. A. B. Luckhardt and his co-workers, and kept free from parathyroid tetany by the oral administration of calcium lactate according to the method described by Luckhardt et al. (1923). Before the administration of desiccated thyroids it required 1 gram to 1.3 gram of calcium lactate per kilo of body weight per day to control parathyroid tetany in this dog, when fed on the high protein dietary described for the dogs of the metabolism series (Kunde and Steinhaus, 1926). After pronounced signs of hyperthyroidism were apparent (11th day of thyroid feeding) the calcium lactate was discontinued and during the following three months no parathyroid tetany has occurred. During this time the dog has received 5 grams of thyroid daily.

SUMMARY

No significant change occurs in the basal metabolism for 7 to 12 hours after the administration of a single dose of Kendall's thyroxin (intravenously) or desiccated thyroids (orally). Following this, an appreciable in-

crease in the heat production occurs which is most marked on the 2nd day.

After daily repeated doses of either desiccated thyroids or Kendall's thyroxin, the basal metabolism progressively increases, reaching a maximal three weeks or more after administering the initial dose.

The maximal increase in the heat production resulting from experimental hyperthyroidism is 120 per cent above normal in the dog with intact thyroid glands and 170 per cent above normal in the previously thyroidectomized animal. This rise in metabolism is always accompanied by fever.

Tachycardia and a disturbance in the conductive mechanism of the heart may both occur as a result of induced hyperthyroidism in the dog.

The influence on the body weight of experimental hyperthyroidism depends entirely on an undetermined nutritional state. Some dogs display a peculiar ability to retain a constant body weight during several weeks of advanced hyperthyroidism on a previously determined maintenance diet. Others may lose 35 per cent of their initial body weight.

Diarrhea, tenesmus and bloody stools may occur as a result of hyperthyroidism.

Severe skin lesions of an eczematous character may occur in some dogs during the crisis of hyperthyroidism.

Exophthalmos cannot be demonstrated in the dog, but in the rabbit the administration of thyroid substance results in a pronounced exophthalmos, provided the rabbit has previously been in a myxedematous condition due to thyroidectomy.

Hyperglycemia and glycosuria do not occur in the dog during experimental hyperthyroidism.

The pathological changes in the thyroid gland during advanced hyperthyroidism consist essentially in a generalized disintegration of the gland with elongated follicular cells containing oval nuclei, and fibroblastic in appearance.

Iodine injected intravenously in the form of potassium iodine does not reduce the basal metabolism of normal dogs. In the dog iodine in the form of Lugol's solution is ineffective in reducing the high basal metabolism or in preventing the metabolism from increasing during the ingestion of thyroid substances.

Our results fail to support the theory that some of the clinical symptoms of either hyperthyroidism or exophthalmos are due to a perverted secretion, since all of the cardinal symptoms of a disturbance in the functional activity of the thyroid gland, excepting cretinism and myxedema, can be produced in either the dog or the rabbit under certain conditions, by the administration of thyroid substances obtained from the glands of normal animals.

A quantitative relationship between the amount of thyroid substance

ingested and the increase in the basal metabolism does not exist, neither is the catalytic theory of Plummer and Boothby adequate for explaining the results of the present experiments on hyperthyroidism.

The degree of toxicity resulting from the ingestion of a given amount of thyroid substance varies markedly with different animals of the same species and depends on some condition of the cells, which at present seems closely related to water metabolism and dehydration phenomena. The thyroidectomized dog and rabbit are more susceptible to the ingestion of thyroid substances than animals with thyroid glands intact.

In a thyro-parathyroidectomized dog, in a tetanoid state, the administration of thyroid substances exerts a marked influence on the control of parathyroid tetany.

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